FACULDADE DE ENGENHARIA DA UNIVERSIDADE DO PORTO

Biomechanical Analysis of Human Movement and Postural Control based on Multifactorial Correlation and Clinical Implications

(Análise Biomecânica do Movimento Humano e do Controlo Postural baseada em Correlação Multifatorial e Implicações Clínicas)

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Biomechanical Analysis of Human Movement and of Postural Control based on Multifactorial Correlation and Clinical Implications

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ABSTRACT

The human body is an inherently unstable system that provides a particularly challenging balance task to the central nervous system, being human walking and standing essentially balance actions. The activation of lower limbs' muscles is in constant need of adaptation to keep a stable and efficient gait and standing, both partially involving similar postural control mechanisms.

This PhD project focuses two domains of motor control. The first is related to bilateral lower limbs movement organisation in gait stance subphases related to higher movement and postural control demand, considering its impact on the metabolic cost of walking. The second is related to standing postural control reorganisation in response to altered afferent input induced by altered support base stability. This twofold objective was explored in nine articles and a book chapter produced within the scope of this PhD thesis. The first article presents a review on biomechanical and neurophysiological mechanisms related to movement and postural control. The book chapter and the second article are related to methodological questions regarding the experimental protocol implemented. The third, fourth and fifth articles are related to the movement organisation purpose. Finally, the sixth, seventh, eighth and ninth articles are related to the postural reorganisation purpose.

Specifically, the first purpose concerning movement organisation was to study the interlimb coordination during gait step-to-step transition. This mechanism was investigated in healthy subjects (n=57) and in subjects with supraspinal damage of structures related to ipsilateral postural control, contra-lateral movement control and interlimb relation resulting from stroke (n=16). The electromyographic activity of both lower limbs and ground reaction force were acquired and were used to calculate the relative magnitude of muscle activity, magnitude of ground reaction force, mechanical work and power over the body´s centre of mass (CoM) during the stance phase of walking. The VO_2 consumption was used to assess the metabolic energy power during walking. The results obtained indicate the existence of a consistent and reciprocal interlimb influence during step-to-step transition in healthy subjects that is mainly related to forward progression - the task more associated to metabolic energy expenditure during walking. In subjects with supraspinal damage at the

internal capsule level[1] resultant from stroke in the middle cerebral artery territory, the interlimb relation is perturbed. The results obtained demonstrate that: 1) only the ipsilateral limb to the affected hemisphere influenced the activity of the contra-lateral limb (paretic limb), and 2) the lower contribution of the paretic limb in forward propulsion is related to a dysfunctional influence of the non-paretic limb during double support. These findings suggest that the lower motor performance of the paretic limb in forward propulsion is not only related to the contra-lateral supraspinal damage but also to a negative influence of the non-paretic limb, probably resulting from a dysfunction of structures ipsilaterally disposed, responsible for postural control.

The second purpose, regarding postural control reorganisation, was to investigate the influence of prolonged exposure to an unstable support base (Masai Barefoot Technology) during daily activities in postural control mechanisms and its impact on systems whose action is closely related to the action of the musculoskeletal system: the peripheral venous system. The electromyographic activity of the trunk and of lower limb muscles, the ground reaction force and the venous cross-sectional area and venous velocity were acquired in a sample of healthy subjects (n=32), before and after a period of unstable shoe wearing. The signals and variables collected were used to calculate individual and muscle group activity, muscle synergies and muscle latencies, centre of pressure (CoP) related parameters, CoM and CoP relation and the venous flow rate. The results indicate that exposure to the instability provided by the kind of shoes adopted leads to positive effects over the postural control that seem to be related to higher acuity muscle spindles. Changes in ankle muscle activity favour peripheral venous circulation even after training adaptation by the neuromuscular system.

Key words: Movement organisation, step-to-step transition, walking, postural control reorganisation, unstable support surface, standing.

[1] Is a flattened band of white fibers located at a subcortical level that contains ascending and descending tracts. The posterior leg of the internal capsule is irrigated by middle cerebral artery and contains corticospinal and corticorreticular pathways.

RESUMO

O corpo humano é um sistema inerentemente instável, que exige ao sistema nervoso central a desafiante tarefa de manutenção do equilíbrio, sendo a marcha humana e a posição de pé na sua essência atos de equilíbrio. A ativação muscular dos membros inferiores está associada a uma necessidade constante de adaptação para garantir estabilidade e eficiência nestas duas tarefas funcionais, que envolvem mecanismos de controlo postural em parte semelhantes.

Este projeto de Doutoramento centrou-se em duas áreas importantes do controlo motor. A primeira está relacionada com a organização bilateral do movimento em subfases de apoio da marcha associadas a uma maior exigência do ponto de vista do movimento e controlo postural, considerando o seu impacto no dispêndio energético metabólico da marcha. A segunda está relacionada com a reorganização do controlo postural na posição de pé em resposta a alterações do *input* aferente, induzido pela diminuição da estabilidade da base de suporte. Este duplo objetivo foi explorado em nove artigos e um capítulo de livro desenvolvidos durante o projeto. O primeiro artigo apresenta uma revisão dos mecanismos neurofisiológicos e biomecânicos relacionados com o movimento e controlo postural. O capítulo de livro e segundo artigo estão relacionados com questões metodológicas importantes na definição dos protocolos experimentais implementados. O terceiro, quarto e quinto artigos focam a organização do movimento. Finalmente, o sexto, sétimo, oitavo e nono artigos focam a reorganização do controlo postural.

Especificamente, o primeiro objetivo relacionado com a organização do movimento foi dirigido ao estudo da coordenação entre membros durante a transição entre passos. Este mecanismo foi investigado em indivíduos saudáveis (n=57) e em indivíduos com história de lesão de estruturas subcorticais intervenientes no controlo postural ipsilateral, no movimento contra lateral e no controlo da relação entre membros, resultante de acidente vascular encefálico (n=16). O sinal eletromiográfico e da força de reação do solo bilaterais foram usados para calcular a magnitude relativa da atividade muscular e da força de reação do solo e o trabalho e potência mecânica realizada sobre o centro de massa (CoM) durante a fase de apoio da marcha. O consumo de VO_2 foi usado para quantificar a taxa de consumo energético metabólico durante a marcha. Os resultados obtidos demostram que os indivíduos saudáveis apresentam uma relação entre membros consistente e recíproca durante a transição entre passos maioritariamente associada à tarefa energeticamente mais

dispendiosa durante a marcha – progressão anterior. Em sujeitos com história de lesão a nível da cápsula interna[2] no território da artéria cerebral média, a relação entre membros encontra-se alterada. Os resultados obtidos demonstram que: (1) apenas o membro ipsilateral ao hemisfério afetado (não parético) influenciou a atividade do membro contra lateral (parético), e (2) a baixa contribuição do membro parético na propulsão anterior está relacionada com uma influência disfuncional do membro não parético durante a fase de duplo apoio. Estes achados sugerem que a diminuição do desempenho motor do membro parético na propulsão está relacionado não apenas com a lesão supraespinal do hemisfério contra lateral, mas também com uma influência negativa do membro não parético, provavelmente resultante da disfunção de estruturas com distribuição ipsilateral responsáveis pelo controlo postural.

O segundo objetivo, relacionado com a reorganização do controlo postural, centrou-se na investigação da influência da exposição prolongada à instabilidade da base de suporte (Masai Barefoot Technology) durante as atividades da vida diária nos mecanismos de controlo postural e o seu impacto em sistemas cuja ação está intimamente relacionado com a ação do sistema musculosquelético: o sistema venoso periférico. A atividade eletromiográfica de músculos do tronco e membro inferior, a força de reação do solo, a área de secção transversal venosa e velocidade venosa foram recolhidas numa amostra de indivíduos saudáveis (n=32), antes e após um período de utilização de calçado instável. As variáveis recolhidas foram utilizadas para calcular a magnitude da atividade muscular individual e sinergias musculares, a latência muscular, parâmetros relacionados com o centro de pressão (CoP) e com a relação entre o CoM e CoP e a taxa de fluxo venoso. Os resultados indicam que a exposição à instabilidade originada pelo tipo de calçado adotado provocou efeitos positivos sobre o controlo postural, o que parece estar relacionado com uma maior sensibilidade do fuso neuromuscular. As alterações ocorridas na atividade dos músculos da tibiotársica mostraram-se vantajosas para a circulação venosa periférica mesmo após a adaptação por parte do sistema neuromuscular.

Palavras-chave: Organização do movimento, transição entre passos, marcha, reorganização do controlo postural, superfície de apoio instável, posição de pé.

[2] É uma banda achatada de fibras brancas situadas a nível subcortical que contém vias ascendentes e descendentes. A parte posterior da cápsula interna é irrigada pela artéria cerebral média e contém as vias corticoespinal e corticorreticular.

CONTENTS

PART A - *THESIS OVERVIEW*

1. INTRODUCTION

Postural control is an essential function in daily activities, which varies as a result of the task-individual-environment interaction (Allum et al., 1998; Shumway-Cook & Woolacott, 2007). Indeed, considering that the body centre of mass (CoM) is located at two-thirds of body height above the ground, the human body is an inherently unstable system that provides a particularly challenging balance task to the central nervous system (CNS) (Winter, 1995). In turn, the CNS has to manage the redundancy of degrees of freedom resulting from the large number of muscles and joints involved to create flexible synergies according to task specificities (Allum et al., 1993, 1994; Diener, 1988; Horak & Nashner, 1986; Keshner, 1988; Macpherson, 1994). The neural process involved in postural control is necessary for all dynamic motor actions (Massion, 1998). In fact, most voluntary movement induces a postural perturbation because of dynamic, inter-segmental forces, and also shifts of the CoM. Therefore, voluntary movements may be considered self-inflicted postural perturbations that may be predicted, to a certain degree, by the CNS, which adjusts the activity of postural muscles both prior to the actual perturbation and in response to it (Arruin, 2002). Two different views have been proposed to explain how the CNS manages both movement and postural control. The first considers two descending control pathways, one accounting for movement control and the other for balance maintenance (Massion, 1992). The second considers the existence of a common controller for focal and postural commands (Aruin & Latash, 1995a, 1995b; Latash, Aruin, & Shapiro, 1995). According to the latter, changes in the activity of postural muscles are not an addition to but an inherent part of a control process for an action (Latash, 1998). In this perspective, complex movements such as gait are a considerable challenge to our understanding, justifying the development of studies in neurophysiological and biomechanical domains, because a large number of segments is involved and many tasks are taking place simultaneously: balance, body support and forward progression, some collaborative and other competitive (Winter & Eng, 1995).

The main argument for differentiating between the balance task during standing and walking is that in walking the CoM does not stay within the support base of the feet leading to a higher and continuous challenge of balance (Kang & Dingwell, 2006; Shumway-Cook & Woolacott, 2007; Winter, 1995; Yang et al., 1990). Despite this, both tasks have been modeled according to an inverted pendulum (Fitzpatrick, Taylor, et al., 1992; Gatev et al., 1999; Horak & Nashner, 1986; Loram & Lakie, 2002b; Runge et al., 1999; Winter, 1995, 2005; Winter et al., 1998), although they require coordination of all joints along the kinematic chain to keep the CoM within safe limits of base of support (Freitas et al., 2009; Jacobs, 1997; Kiemel et al., 2008; Morasso & Schieppati, 1999; Nicholas et al., 1998; van der Kooij et al., 1999), and in both, postural control acts to control the CoM as to the environment conditions[3] (Dietz et al., 1992; Latash, 1998). Similar postural control strategies have been demonstrated in the stance phase of walking and in standing, although only for the first half stance, whose mechanical conditions are quite different from those of late single-support (Yang, et al., 1990). A less significant role in postural control during walking was observed in the swing leg (Yang, et al., 1990), which could explain the minimal muscular power output when compared to the output during stance (Morasso et al., 1999; Neptune, Kautz, et al., 2004).

Studies on balance and posture during quiet or perturbed standing have identified the dominance of ankle muscles in the anteroposterior direction and hip abd/adductor muscles in the mediolateral direction (Fitzpatrick, Taylor, et al., 1992; Johanson, 1993; Kuo, 1993; Winter, 1995; Winter et al., 1996). During quiet standing, postural sway results from a combination of inherent fluctuations in the musculoskeletal system, cardiac and respiratory variations, and neural activity. However, it has also been suggested that postural sway serves as an exploratory behavior for the stimulation of somatosensory and vestibular pathways to provide sensory information for increased postural control (Riley et al., 1999; Riley et al., 1997). By reconciling these two points of view, it is likely that sway characteristics result from an interaction between physiological states, the environment, and the implicit and explicit goals of the current task (Rothwell, 1994; Shumway-Cook & Woolacott, 2007).

Under normal conditions during standing on a rigid floor the postural control system elaborates the reference position using information about the relative positions of body segments, muscular torques and interaction with the base of support, taking into account the energy cost of standing and demands for stability and orientation (Dietz, 1992; Gurfinkel et al., 1995; Horak et al., 1990). The body alignment can minimise the effect of gravitational forces that tend to pull-off the CoM from the base of support, and muscle tone (intrinsic stiffness of the muscles, background muscle tone and postural tone) keeps the

[3] Such as the gravitational forces, the reaction forces from the supporting surfaces, imposed accelerations and obstacles.

body from collapsing due to gravity (Shumway-Cook & Woolacott, 2007). When postural conditions change, the CNS must identify and selectively focus the most reliable sensory inputs to provide optimal control. As a result of this weighting of afferent input, muscle forces can be produced to control the CoM efficiently to maintain a good equilibrium (Carver et al., 2006). Much of the research has focused in quantifying the human response to balance system perturbation in a different number of ways, such as displacement of support surface, predictable and unpredictable external perturbations and internal perturbations (Aruin & Latash, 1995a, 1995b; Aruin et al., 2001; Friedli et al., 1988; Mouchnino et al., 1992; Nashner, 1976, 1982; Pedotti et al., 1989; Rogers & Pai, 1990; Santos et al., 2009; Winter et al., 1993; Wolfson et al., 1986). Several factors have been shown to influence postural control responses, but only at an immediate level. Considering the high adaptability of the CNS (Winter, 1984) in response to changing task and environment demands (Shumway-Cook & Woolacott, 2007), further investigation is required regarding the long-term influence of these changes of afferent information that could be beneficial to postural control. Given the importance of the ankle joint in upright postural control (Diener et al., 1984; Fitzpatrick et al., 1994; Fitzpatrick, Taylor, et al., 1992; Kavounoudias et al., 2001), the influence of an unstable support base in postural control (Dietz et al., 1980; Gantchev & Dimitrova, 1996; Gavrilenko et al., 1995; Ivanenko et al., 1999) is of significant relevance. When standing on an unstable support base the new postural requirements lead to postural control reorganisation through increased central drive (Gavrilenko, et al., 1995; Ivanenko, et al., 1999) associated with augmented gamamotoneuron activity leading to higher sensitivity of the muscle spindles (Dietz, et al., 1980; Gorassini et al., 1993; Prochazka, 2010; Ribot-Ciscar et al., 2000) and higher muscle co-contraction (Dietz, et al., 1980). However, anticipatory postural adjustments (APA) have been shown to decrease not only in very stable conditions (Nardone & Schieppati, 1988) but also in very unstable conditions (Arruin et al., 1998; Gantchev & Dimitrova, 1996; Nouillot et al., 1992). Since the need for stabilising posture is diminished in stable conditions, the requirement for APA is also reduced (Nardone & Schieppati, 1988). Also, APA could themselves be a potential source of perturbation in case of unstable posture and as such they are also reduced not to additionally destabilise posture (Arruin, et al., 1998). In this sense, it seems that the permanence in an unstable support base could, up to a certain level of instability, improve postural control. Although there is extensive research regarding the impact of changes in support surface base, all these studies either addressed the immediate response (Dietz, et al., 1980; Gantchev & Dimitrova, 1996; Gavrilenko, et al., 1995; Ivanenko, et al., 1999; Nigg, Hintzen, et al., 2006) or the long-term effect resulting from controlled training conditions in unstable surfaces (Taube et al., 2008; Turbanski et al., 2011). To the best of our knowledge, no study has assessed in a comprehensive way the long-term influence of prolonged standing in an unstable support base, such as in daily activities, on postural control. Recently, some studies have assessed the influence of prolonged standing in an unstable support base on a few variables related to postural control (Landry et al., 2010; Nigg, Emery, et al., 2006; Ramstrand et al., 2008), which is not enough for understanding the impact of this kind of change in afferent input in postural control (Pavol, 2005). This is an important area of research in rehabilitation and motor performance, which can give significant insights concerning not only motor control but also areas that have been dedicated to the implementation of preventive measures, like Ergonomy. In this last perspective, the influence of postural control reorganisation in response to changes in afferent input, in systems that are dependent from the action of the musculoskeletal system, must also be further analysed.

The importance of ankle muscles in balance during walking is less determinant because a forward fall of CoM is required to accelerate the CoM ahead of the base of support (Winter, 1995) which is determined by the vector joining the CoP and the CoM during single-support (Winter, 2005). Only the safe placement of the swing foot prevents a fall every step and restabilisation can take place during the two double support periods. Also, the body lateral motion is partially stabilised via medio-lateral foot placement (Donelan et al., 2004) during double support. However, the support base is not very firm during this time since one foot is accepting weight on the small area of the heel while the other is pushing off on the forepart of the foot (Winter, 1995; Yang, et al., 1990). Consequently, from a postural control perspective, the double support is an important phase of walking that depends on the activity of each limb but also on the synergy between them. The importance of this subphase is enhanced by the relation between postural stability and the metabolic cost of walking (Donelan, et al., 2004; Holt et al., 1995). From a movement control side, the double support phase has been described as one of the most determinant concerning its high impact on the metabolic cost of walking (Donelan et al., 2002a). The major cause of mechanical inefficiency during healthy gait is the generation of energy at one joint and the absorption at another joint which occurs mainly during the double support phase of gait. Indeed, the energy increase of the push-off leg takes place as the weight-accepting leg absorbs energy (Winter, 2005). Also, to redirect and accelerate the CoM from step-to-step transition, the amount of energy lost to each collision between the foot and the ground at heel-strike must be replaced by contra-lateral limb propulsion (Donelan, et al., 2002a; Kuo et al., 2005, 2007; Neptune, Zajac, et al., 2004) to redirect the CoM upward and forward (Donelan, et al., 2002a). In fact, it has been demonstrated that the work performed to propel the CoM forward during final stance constitutes nearly one-half of the net metabolic cost of normal walking (Donelan, et al., 2002a; Gottschall & Kram, 2003; Grabowski et al., 2005).

Besides the work to redirect and accelerate the CoM from step-to-step transition, the metabolic cost of walking is also determined by the generating force to support body weight (Cavagna et al., 1976; Donelan & Kram, 1997; Donelan, et al., 2002a; Donelan et al., 2002b; Donelan, et al., 2004; Gottschall & Kram, 2003; Griffin et al., 2003; Griffin et al., 1999). The contribution of body weight support to the cost of walking during the single-stance phase has been explained on the basis of the inverted pendulum model (Cavagna et al., 1977). Although this model suggests that little mechanical work must be performed on the CoM (Cavagna, et al., 1976), the existence of a nearly isometric muscular force to prevent the leg from collapsing and to support the weight of the body has recently been shown (Grabowski, et al., 2005). However, despite incurring a significant metabolic cost (Grabowski, et al., 2005), performing isometric force is only one-half of the cost of generating active work during step-to-step transition.

Despite the importance of the double support phase in the metabolic cost of walking, in the perspective of postural and movement control the interlimb coordination has been poorly explored. This can probably be explained by the fact that healthy subjects walking is an energy-cheap activity (Saibene & Minetti, 2003), as interlimb coordination is perfectly integrated. However, this interlimb coordination is regulated by supraspinal (Davies & Edgley, 1994; Matsuyama et al., 2004) and spinal mechanisms (Bajwa et al., 1992; Corna et al., 1996; Dietz, 1992) that could be impaired directly or indirectly as a consequence of lesions in specific supraspinal neuronal structures (Fries et al., 1993; Marque et al., 2001; Maupas et al., 2004; Nardone & Schieppati, 2005). Consequently, the study about the interlimb coordination in healthy subjects is needed to understand the atypical behaviour in pathology, particularly in individuals presenting asymmetric lower limbs motor impairment as post-stroke subjects. Such knowledge has the potential to provide a foundation for answering clinical questions pertaining to how the movement control system changes for specific cases, how to look for alterations in performance, what changes to look for during the analysis of movement, and most importantly how outcomes of interventions may be quantified.

The importance of this research area is highlighted by the notion that gait energy efficiency may be impaired in stroke subjects due to the reduction in strength, but also due to the reduced coordination of the affected leg or legs (Kuo & Donelan, 2010). Also, additionally to the causes of mechanic inefficiency described for healthy gait, in stroke gait there are other factors responsible for mechanical inefficiency like increased co-contraction and isometric contractions against gravity (Winter, 2005). Biomechanical characteristics of stroke walking have been vastly explored (Chen et al., 2005b; Goldie et al., 2001; Kim & Eng, 2004; Lamontagne et al., 2002; Lamontagne et al., 2000; Lamontagne et al., 2007; Lin et al., 2006; Olney et al., 1994; Olney & Richards, 1996; Shiavi et al., 1987b; Verma et al., 2012; Woolley, 2001). However, to the best of our knowledge no study has assessed the interlimb coordination during step-to-step transition of walking.

This PhD project focused mainly the study of postural control reorganisation in stance-related activities in response to altered afferent input induced by altered support base stability conditions and of movement organisation in gait stance subphases related to higher movement and postural control energy demand. Bernstein predicted that CNS control is applied at the level of joint moments or at the synergy level when he postulated the "principle of equal simplicity" as "it would be incredibly complex to control each and every individual muscles" (Bernstein, 1967). All the work developed within the scope of this thesis is based on this principle.

2. MAIN OBJECTIVES

The key objectives defined for this PhD project were organised around the assessment of two main domains of motor control: movement organisation and postural control reorganisation. As such, the central objectives addressed can be stated as follows:

- to evaluate the bilateral lower limb movement organisation in functional tasks of high movement and postural control demand, like the step-to-step transition of walking;

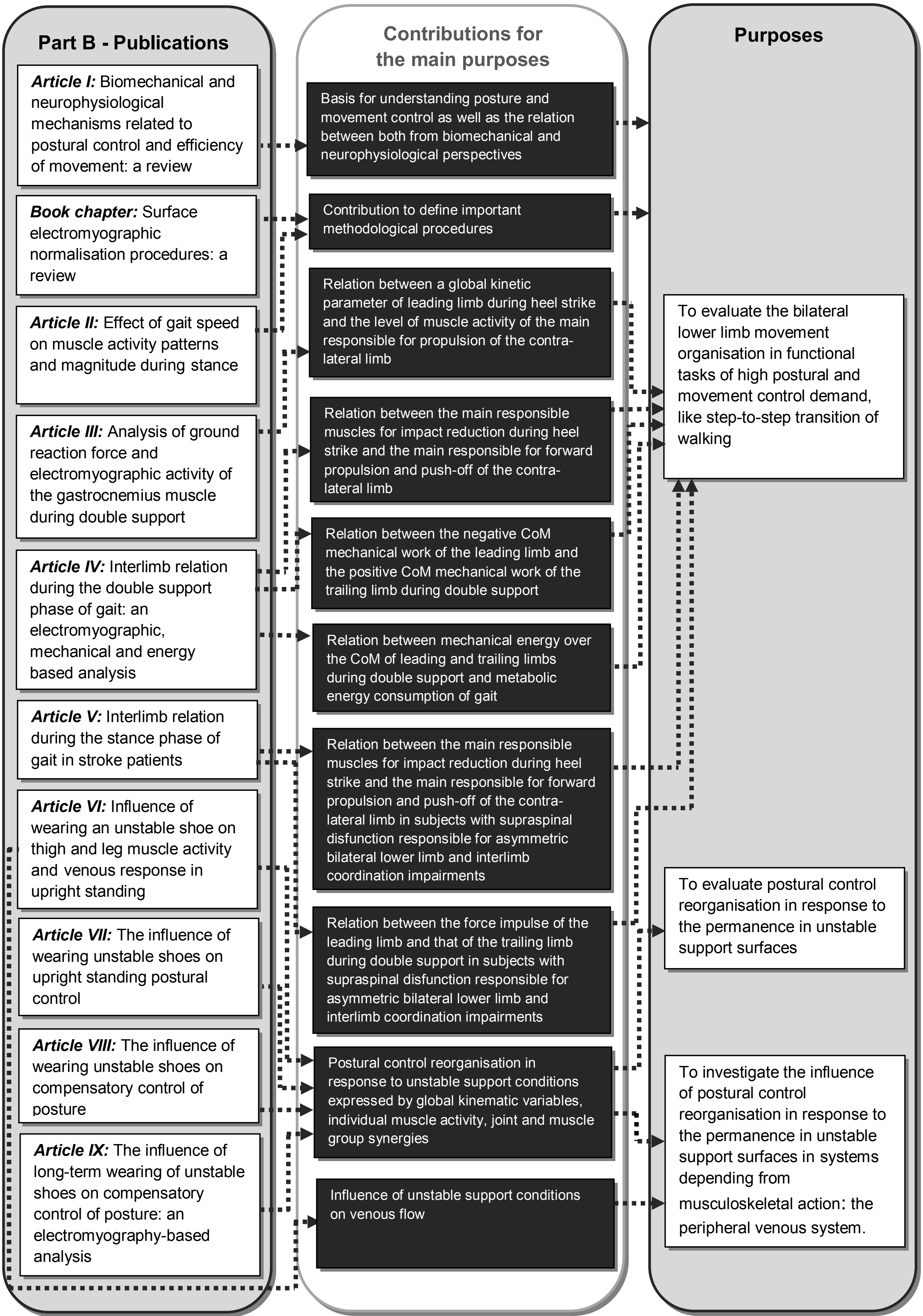
- to evaluate postural control reorganisation in response to the permanence in an unstable support base during daily activities;

- to investigate the influence of postural control reorganisation in response to the permanence in an unstable support base in systems closely related to musculoskeletal action – the peripheral venous system.

To assess the purposes above, musculoskeletal dynamics, expressed by kinematic, kinetic and electromyographic factors, and energy expenditure were investigated. To evaluate the bilateral lower limb movement organisation, the interlimb coordination was quantified in healthy subjects and in subjects with supraspinal lesion of structures related to interlimb coordination, movement and postural control. The results obtained were interpreted as to their clinical implications, taking into account neurophysiological and biomechanical principles.

3. THESIS ORGANISATION

This PhD thesis consists of two mutually dependent parts. Part A presents an overview of the research developed and Part B contains publications that describe and discuss the research results in more detail. Specifically, the following four sections of this Part A are reserved to expose briefly the work carried out. Hence, the work undertaken is briefly presented in the next section, including the motivations and objectives, methodological procedures adopted, main results obtained, respective conclusions and contributions of each publication included in Part B. This section is followed by an overall discussion of the results obtained on whole work undertaken (section 5). Then, in section 6, the main contributions achieved during this PhD thesis are indicated and the main conclusions and future work perspectives are stated in section 7. Part B includes nine journal articles and one book chapter, selected for their contribution to the research objectives of this thesis. The organisation of the work undertaken as well as the contributions of each publication are depicted in the following diagram.


Part B - Publications
Contributions for the main purposes
Purposes
Article I: Biomechanical and neurophysiological mechanisms related to postural control and efficiency of movement: a review
Book chapter: Surface electromyographic normalisation procedures: a review
Article II: Effect of gait speed on muscle activity patterns and magnitude during stance
Article III: Analysis of ground reaction force and electromyographic activity of the gastrocnemius muscle during double support
Article IV: Interlimb relation during the double support phase of gait: an electromyographic, mechanical and energy based analysis
Article V: Interlimb relation during the stance phase of gait in stroke patients
Article VI: Influence of wearing an unstable shoe on thigh and leg muscle activity and venous response in upright standing
Article VII: The influence of wearing unstable shoes on upright standing postural control
Article VIII: The influence of wearing unstable shoes on compensatory control of posture
Article IX: The influence of long-term wearing of unstable shoes on compensatory control of posture: an electromyography-based analysis
Basis for understanding posture and movement control as well as the relation between both from biomechanical and neurophysiological perspectives
Contribution to define important methodological procedures
Relation between a global kinetic parameter of leading limb during heel strike and the level of muscle activity of the main responsible for propulsion of the contra-lateral limb
Relation between the main responsible muscles for impact reduction during heel strike and the main responsible for forward propulsion and push-off of the contra-lateral limb
Relation between the negative CoM mechanical work of the leading limb and the positive CoM mechanical work of the trailing limb during double support
Relation between mechanical energy over the CoM of leading and trailing limbs during double support and metabolic energy consumption of gait
Relation between the main responsible muscles for impact reduction during heel strike and the main responsible for forward propulsion and push-off of the contra-lateral limb in subjects with supraspinal disfunction responsible for asymmetric bilateral lower limb and interlimb coordination impairments
Relation between the force impulse of the leading limb and that of the trailing limb during double support in subjects with supraspinal disfunction responsible for asymmetric bilateral lower limb and interlimb coordination impairments
Postural control reorganisation in response to unstable support conditions expressed by global kinematic variables, individual muscle activity, joint and muscle group synergies
Influence of unstable support conditions on venous flow
To evaluate the bilateral lower limb movement organisation in functional tasks of high postural and movement control demand, like step-to-step transition of walking
To evaluate postural control reorganisation in response to the permanence in unstable support surfaces
To investigate the influence of postural control reorganisation in response to the permanence in unstable support surfaces in systems depending from musculoskeletal action: the peripheral venous system.

4. DESCRIPTION OF THE WORK DEVELOPED

This section provides a briefly description of the work developed during this PhD project to address the main objectives defined. The work developed is presented in detail throughout the nine articles and the book chapter included in Part B.

4.1 State-of-the-art review

The study of movement and postural control requires understanding biomechanical and neurophysiological related mechanisms. Neurophysiologists tend to focus on the principles of organisation of the central networks that generate muscle activity patterns. On the other side, biomechanical researchers focus mainly on movement mechanics, including limb and body kinematics, kinetics and energy cost. Although complementary, these two approaches have not been frequently associated. In fact, the conclusions obtained in each field could contribute more significantly to the construction of scientific knowledge. In an attempt to explore a link between the two approaches, a review of biomechanical and neurophysiological mechanisms related to postural control and efficiency of movement was performed (Sousa, Silva, & Tavares, 2012a) - *Article I* (Part B). Hence this article reviews the main concepts associated to this PhD project.

Title: Biomechanical and neurophysiological mechanisms related to postural control and efficiency of movement: a review

Authors: Andreia S. P. Sousa, Augusta Silva, João Manuel R. S. Tavares

Journal: Somatosensory and Motor Research (2012), 29(4):131-143

Brief description: This article is an introduction to the subject developed in this project. The main purpose is to summarise the functional relation between biomechanical and neurophysiological perspectives related to postural control in both standing and walking based on movement efficiency. For this purpose, evidence related to the biomechanical and neurophysiological mechanisms is explored, as well as the role of proprioceptive input on postural control and movement control. This article provides the notion that the complexity of the interrelations between neural and mechanical aspects and the environment leads to the need of studying postural control in a comprehensive way, and concludes that the study of postural control needs to reflect the dynamic interrelation of the different components of human movement on the basis of movement efficiency.

The electromyography assumed an important role in assessing the purposes of this project as it was used to quantify, besides other parameters, the degree of muscle activity in all experimental work undertaken. Considering that the comparison of the level of electromyographic activity between different muscles, across time and between individuals requires signal normalisation and that several options have been presented in related research studies, a review of surface electromyographic amplitude normalisation methods was accomplished and presented in (Sousa & Tavares, 2012b) – *Book chapter* (Part B):

Title: Surface electromyographic amplitude normalisation methods: a review

Authors: Andreia S. P. Sousa, João Manuel R. S. Tavares

Book: Electromyography: new developments, procedures and applications

Publisher: Neuroscience Research Progress, Nova Science Publishers, Inc., Chapter V, pp. 85-101, 2012

Brief description: This chapter presents a review of the most applied normalisation methods. The main purpose is to discuss the implications of each amplitude normalisation method based on advantages and disadvantages/limitations, namely regarding reliability, variability, ability to detect changes in external force and physiological meaning, and on its influence on data interpretation. Several studies comparing the different methods were reviewed, indicating that isometric and dynamic methods (mean dynamic method and peak dynamic method) are the most recommended. However, both present advantages and limitations and their application is dependent on the subjects' neuromusculoskeletal condition. In addition it is also important to choose the appropriate normalisation technique according to the purpose of the electromyographic study since this choice will change outcome measures and subsequent data interpretation.

The review as to the methods for electromyographic signal normalisation supported the decisions adopted in the work briefly exposed in the following sections.

4.2 Movement organisation during the stance phase of gait

As stated in the introduction section, part of this project was dedicated to the study of movement organisation in the stance phase of gait. The first study was related to the work undertaken in the MSc project and was implemented to define in a sustained way the most appropriate gait speed to be adopted in gait studies assessing electromyographic activity magnitude. This is an important area of research because in general changes in walking speed require the adjustment of a neuromuscular gain factor while the global timing characteristics of muscle activity patterns are essentially preserved (Cappellini et al., 2006; den Otter et al., 2004; Hof et al., 2002; Shiavi et al., 1987a). The importance of this study for this PhD project is related with the definition of the speed protocol adopted, because most studies addressing the effect of gait speed on electromyography parameters used the same standard speeds for all subjects and focused the effect on muscle timing pattern (den Otter, et al., 2004; Hof, et al., 2002). Consequently, there are questions as to the most correct gait speed to evaluate muscle activity magnitude: (1) selecting a standard speed to be adopted by all subjects or (2) not restricting gait speed, allowing subjects to walk at their self-selected speed. To the best of our knowledge no study analysed the influence of self-selected speed variation on gait muscle activity levels and magnitude patterns. This analysis gives insights into which method would represent more accurately gait muscle activity patterns in terms of amplitude. Also, this study provides a reflexive analysis of the relative role of different lower limb muscles along the stance phase of gait that was useful to works undertaken to assess interlimb relation during gait. This work is presented in (Sousa & Tavares, 2012a) - *Article II* (Part B).

Title: Effect of gait speed on muscle activity patterns and magnitude during stance

Authors: Andreia S. P. Sousa, João Manuel R. S. Tavares

Journal: Motor Control (2012), 16(4):480-492

Brief description: This work aimed to study the impact of speed variations from self-selected walking speed on muscle activity patterns and magnitude during stance. Thirty-five healthy individuals participated in the study. The surface electromyographic activity from muscles responsible for most of the work on the CoM, such as the gastrocnemius medialis (GM), gluteus maximus (GMax), biceps femoris (BF) and rectus femoris (RF), was acquired while subjects walked at three different speeds: one self-selected, other 25%

lower and another 25% higher. The root mean square of the electromyographic signal was calculated to assess the muscle activity level during heel strike, during the transition between heel strike and midstance (loading response), during the transition between midstance and propulsion (push off) and during propulsion (pre-swing), Figure 1.

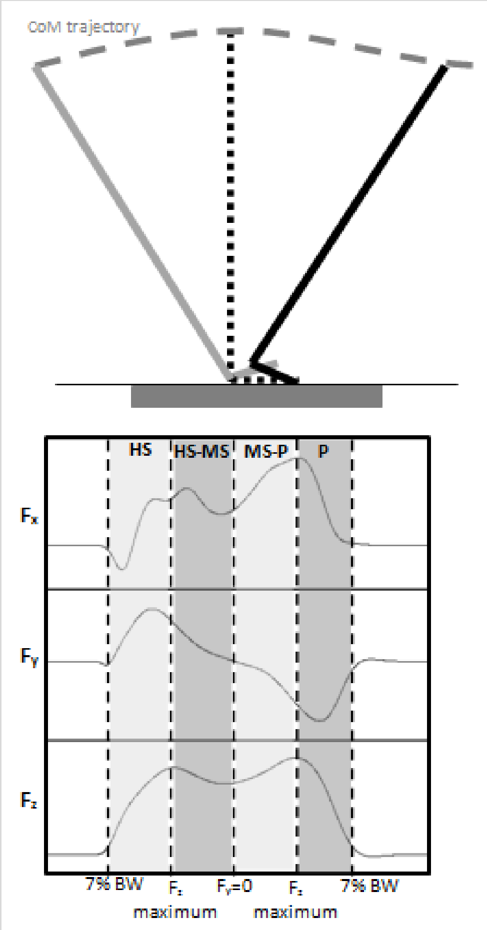


Figure 1: Representation of the criteria adopted to define stance subphases.

As can be observed in Figure 1, the first stance subphase – heel strike (HS) – was defined as the time between the moment that F_z reached a value equal to 7% of body weight (BW) till the moment that F_z reached the first local maximum peak (F_z maximum). The second stance suphase – transition between heel strike and midstance (HS-MS) – was defined as the time between the first F_z maximum till the moment that F_y assumed the value zero (F_y=0). The third stance subphase – transition between midstance and propulsion (MS-P) – was defined as the time between the value of F_y=0 till the moment that F_z reached the second local maximum peak (F_z maximum). The last stance subphase – propulsion (P) – was defined as the time between the second F_z maximum till the moment that F_z reached for the second time the value of 7% BW.

We have compared values of muscle activity obtained at each speed, as well as between each stance subphase, and the results demonstrate that speed variation from self-selected speed leads to: 1) changes in the activity level of RF, GMax, gastrocnemius medialis (GM) and biceps femoris (BF), in a decreasing order and 2) changes in the relative motor activity patterns in almost all stance subphases. The results also demonstrate that muscle activity was higher at the faster and slower speeds than at the self-selected speed, while only the activity of the main actions in each subphase remained stable. These findings suggest that gait speeds different from the self-selected speed influence not only activity levels but also relative muscle activity patterns. As a result, caution is advised when choosing standard speeds in gait studies, as this can lead to increased variability in relative muscle activity patterns.

Considering the results obtained in *Article II* the studies related to the assessment of interlimb relation during the stance phase of walking were carried out at self-selected speed.

The importance of studying interlimb relation during gait is based on the impact of this relation on the metabolic cost of walking (Donelan, et al., 2002a, 2002b; Kuo, et al., 2005, 2007) and is supported by biomechanical (Donelan, et al., 2002a, 2002b; Kuo, et al., 2007; Winter, 2005), and neurophysiological factors at spinal (Bajwa, et al., 1992; Corna, et al., 1996; Dietz, et al., 1992; Nardone et al., 1996) and supraspinal levels (Davies & Edgley, 1994; Drew et al., 2004; Jankowska et al., 2003; Matsuyama, et al., 2004). At supraspinal level the role of cortico-ponto-reticulospinal-spinal interneuronal system in controlling the interlimb relation has been focused (Jankowska, et al., 2003; Matsuyama, et al., 2004) while at spinal levels it has been demonstrated that the neural circuits controlling each leg are coupled (Bajwa, et al., 1992; Nardone, et al., 1996; Reisman et al., 2005). Experiments in animals have demonstrated the existence of a group of interneurons (Edgley & Jankowska, 1987; Edgley et al., 2003; Jankowska & Noga, 1990) that receive supraspinal input from the vestibulo- and reticulo-spinal pathways and pyramidal tract (Davies & Edgley, 1994; Jankowska et al., 2005; Jankowska et al., 2006) and bilateral peripheral input from group Ia, group II (Jankowska, et al., 2005) and cutaneous afferents (Edgley & Aggelopoulos, 2006). Considering all these levels, kinesthetic afferences may be critically involved in sustaining coordination between limbs (Swinnen et al., 1990). The role of spindle group II fibers has been highlighted in humans as, despite being of smaller diameter are as numerous as the group Ia fibers, these may be even more relevant as the origin of information used by postural control circuits (Morasso, et al., 1999; Nardone, Corrà, et al., 1990; Nardone, Giordano, et al., 1990; Schieppati et al., 1995). Some authors go further arguing that both legs and foot muscles are the site of postural control segmental reflexes (Schieppati, et al., 1995) mainly because of spindle group II fibers (Grey et al., 2001; Grey et al., 2002; Nielsen & Sinkjaer, 2002; Schieppati & Nardone, 1997; Sinkjær et al., 2000).

Our first study, related to interlimb relation during gait, aimed to verify if a simple measure like the ground reaction force magnitude during heel strike could predict plantar flexor activity magnitude during contra-lateral limb propulsion. This knowledge is very useful for understanding the impact of one limb on the other from a motor control and biomechanical point of view, but also to provide important guidelines in rehabilitation strategies in subjects with predominant unilateral or bilateral asymmetric dysfunctions. Also, the results of this study could provide useful information for the creation of hybrid orthoses with the capacity of adjusting the activity of one limb according to biomechanical characteristics of the contra-lateral limb. This work is described in (Sousa, Santos, et al., 2012) – *Article III* (Part B).

Title: Analysis of ground reaction force and electromyographic activity of the gastrocnemius muscle during double support

Authors: Andreia S. P. Sousa, Rubim Santos, Francisco P. M. Oliveira, Paulo Carvalho, João Manuel R. S. Tavares

Journal: Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine (2012), 226(5):397-345

Brief description: Mechanisms associated with energy expenditure during gait have been extensively researched and studied. According to the double-inverted pendulum model energy expenditure is higher during double support, as lower limbs need to work to redirect the CoM velocity. This study looked into how the ground reaction of one limb affects the muscle activity required by the GM of the contra-lateral limb during step-to-step transition. Thirty-five subjects were monitored as to the GM electromyographic activity of one limb and the ground reaction force of the contra-lateral limb during step-to-step transition at self-selected speed, Figure 2.

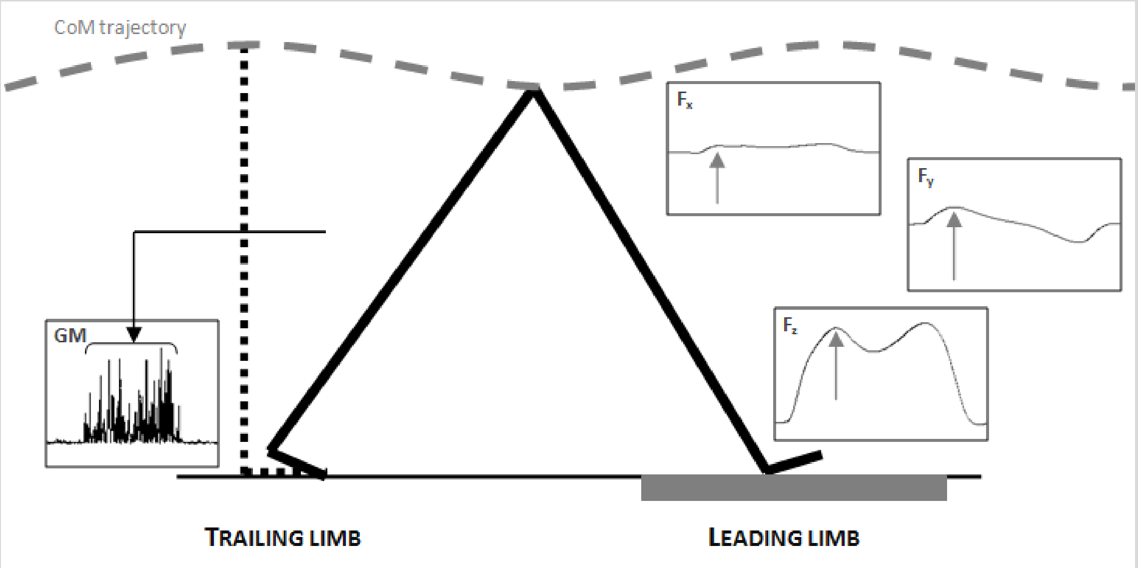


Figure 2: Schematic representation of the protocol used to assess the purpose of this study.

The root mean square of the electromyographic activity of the GM of the trailing limb was calculated in a time window defined as the period between the transfer of load from the calcaneous to the first metatarsal head and the maximum load over the first metatarsal head (assessed by load cells positioned at these anatomic locations).The ground reaction force of the leading limb was collected using a force plate and the value of the first maximum peak of each component (mediolateral (F_x), anteroposterior (F_y) and vertical F_z) was used for analysis.

A moderate correlation was observed between the activity of the GM muscle of the dominant leg and the vertical and anteroposterior components of ground reaction force of the non-dominant leg and a weak and moderate correlation was observed between the GM muscle activity of the non-dominant leg and the vertical and anteroposterior components of ground reaction force of the dominant leg, respectively. The results obtained suggest that during step-to-step transition the ground reaction force is associated with the electromyographic activity of the contra-lateral GM muscle.

A relation between the electromyographic activity of one limb during push-off and the magnitude of the ground reaction force of the other limb during heel strike was found (Sousa, Santos, et al., 2012), which demonstrates that the behaviour of the leading limb affects that of the trailing. However, although influencing directly the CoM (Winter et al., 1990), the peak of ground reaction force measurements represents an instantaneous value and consequently is not representative of the external work developed over the CoM. In addition, it could be argued that a more direct interlimb relation would be observed in terms of muscle activity, considering evidence of the bilateral influence of afferent fibres over midlumbar interneurons recipient from group II input (Bajwa, et al., 1992; Corna, et al., 1996; Dietz, 1992). Taking this into account, the work related to the understanding of interlimb relation during gait continued with the analysis of muscle activity and the parameters most relating to CoM displacement (external mechanical work) and its impact on metabolic energy expenditure. This work is described in (Sousa, Silva, & Tavares, 2012b) – *Article IV* (Part B):

Title: Interlimb relation during the double support phase of gait: An electromyographic, mechanical and energy based analysis.

Authors: Andreia S. P. Sousa, Augusta Silva, João Manuel R. S. Tavares

Journal: Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine (2013); 227(3) DOI: 10.1177/0954411912473398 (in press).

Brief description: The purpose of this study was to analyse interlimb relation in terms of mechanical work over the CoM and electromyographic activity and its relation to metabolic energy expenditure during gait. Twenty two subjects were monitored as to the electromyographic activity of muscles dominating work output over the gait cycle (tibialis anterior (TA), GM, soleus (SOL), BF, RF and vastus medialis (VM) muscles), ground reaction force and VO_2 consumption during gait at self-selected speed. Ground reaction force values were used to calculate the mechanical work performed on the CoM (1) and the mechanical CoM power (mechanical work performed on the CoM divided by body mass (kg) and time (s)) during double support, Figure 3, and the values of VO_2 consumption were used to calculate metabolic power (2). The equations adopted to calculate the parameters mentioned were:

(1) $W_{trail} = \int \vec{F}_{trail} \times \vec{v}_{com},$

$$W_{lead} = \int \vec{F}_{lead} \times \vec{v}_{com},$$

$$\vec{v}_{com} = \int \left(\frac{\vec{F}_{trail} + \vec{F}_{lead} + m \cdot \vec{g}}{m} \right) dt + \begin{bmatrix} C_x \\ C_y \\ C_z \end{bmatrix},$$

where w_{trail} is the mechanical work performed in the CoM by the trailing limb, $\vec{F}_{trail}$ is the ground reaction force vector exerted by the trailing limb, push-off, $\vec{v}_{com}$ is the velocity vector of the CoM, W_{lead} is the mechanical work performed in the CoM by the leading limb, $\vec{F}_{lead}$ is the force exerted by the leading, new stance, m is the participants' body mass, $\vec{g}$ is the gravitational acceleration ([0, 0, -9.81] m.s^{-1}) and C_x, C_y and C_z are the integration constants for the medio-lateral, fore-after and vertical directions, respectively. Integration constants were calculated according to (Donelan, et al., 2002b).

(2) $\dot{E}_{met} = 4.94 \times RER + 16.04 \times VO_2$,

where $\dot{E}_{met}$, is the metabolic energy consumption and RER is the respiratory exchange ratio. The metabolic power was obtained by dividing metabolic energy consumption by body mass (kg).

The root mean square of the electromyographic activity was calculated for the transition between midstance and double support (MS-DS) and for double support (DS), Figure 3. The results obtained demonstrate a moderate negative correlation between the activity of TA, BF and VM of the trailing limb during MS-DS and those of the leading limb during DS and between these last ones and the trailing limb's GM and SOL during DS. The SOL muscle of the trailing limb during MS-DS was positively related to TA, VM and BF of the leading limb during DS. Also, the mechanical work over the CoM by the trailing limb was strongly influenced by that of the leading limb, but only the mechanical power related to forward progression of both limbs was related to metabolic power. These results demonstrate a consistent interlimb relation in terms of electromyographic activity and mechanical work over the CoM, being these relations in the plane of forward progression the most important to gait energy expenditure.

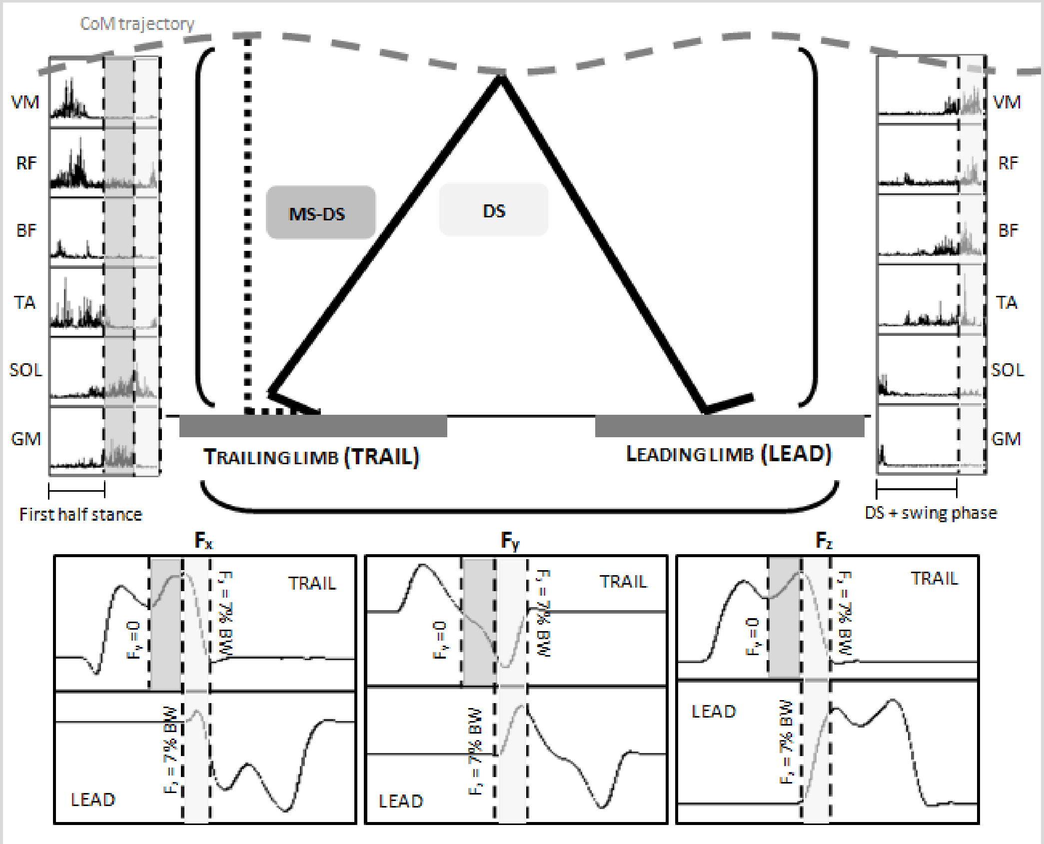


Figure 3: Schematic representation of the protocol used to assess interlimb relation during gait. The variables collected in each limb are presented, as well as the information about the interval used for processing each variable in each limb. It should be noted that the signals are presented using different scales to facilitate the perception of the intervals used for analysis.

Although healthy subjects appear to accomplish economical step-to-step transitions across the full range of walking speed, economy may be impaired in people with gait pathologies such as stroke due to the reduction in strength (force generation capacity) and coordination of the affected leg or legs (Kuo & Donelan, 2010). In patients recovering from stroke, mechanical work measurements suggest that the paretic leg performs much less push-off work and that both legs perform more total mechanical work than that performed by speed-matched individuals who are healthy (Kim & Eng, 2004; Olney et al., 1991; Olney & Richards, 1996). This increase in work suggests that patients recovering from stroke experience an elevated metabolic cost because step-to-step transitions require more mechanical work and not because they perform work less efficiently (Kuo & Donelan, 2010). Considering the importance of step-to-step transition in gait energy expenditure, and the consistent interlimb relation observed in healthy subjects (*Articles III and IV*), the investigation of interlimb relation in terms of electromyographic and kinetic aspects was also carried out in stroke subjects. The importance of assessing interlimb relation in stroke patients relies not only on understanding the impact of the limb contra-lateral to the affected hemisphere (paretic limb) on the ipsilateral limb (non-paretic limb), but also the impact of the non-paretic limb on the paretic limb. This happens because paretic limb performance can be affected by neural pathways responsible for movement control while non-paretic limb performance can be affected by ipsilaterally distributed neural pathways responsible for postural control (Latash, 1998; Matsuyama, et al., 2004) and because changes in the motor function of the non-paretic limb have been documented (Cramer et al., 1997). This work is described in (Sousa, Silva, Santos, et al., 2012) – *Article V* (Part B).

Title: Interlimb coordination during the stance phase of gait in stroke patients

Authors: Andreia S. P. Sousa, Augusta Silva, Rubim Santos, Filipa Sousa, João Manuel R. S. Tavares

Journal: Submitted to an International Journal

Brief description: Interlimb coordination assumes an important role in gait energy consumption. The purpose of this study was to analyse the relation between non-paretic and paretic limbs in stroke subjects during walking. Sixteen post-stroke subjects and twenty two healthy control individuals were monitored as to bilateral electromyographic activity of TA, GM, SOL, BF, RF and VM and ground reaction force during gait. The root mean square of the electromyographic activity and the propulsive impulse assessed from the ground reaction force were calculated for double support and the second half of single support. Two conditions were evaluated during double support: 1) when the paretic limb was the trailing limb and the non-paretic limb was the leading limb and 2) when the paretic limb was the leading limb and the non-paretic limb was the trailing limb, Figure 4. The results obtained indicate that the propulsive impulse of the paretic trailing limb was negatively correlated to the braking impulse of the leading limb during double support. A moderate functional relationship was observed between thigh muscles, and a strong and moderate dysfunctional relationship was found between the plantar flexors of the non-paretic limb and the vastus medialis of the paretic limb. Also, a functional moderate negative correlation was observed between the SOL muscle of the non-paretic limb during transition between midstance and double support (MS-DS) and the SOL of the paretic limb during heel strike and between the RF muscle of the non-paretic limb during MS-DS and the VM muscle of the paretic limb during heel strike. The propulsive impulse contribution of the paretic trailing limb was lower than that of the non-paretic trailing limb in patients with stroke and in healthy subjects. The findings obtained seem to suggest that the lower performance of the paretic limb in forward propulsion during gait is not only related to contra-lateral supraspinal damage, but also to a dysfunctional influence of the non-paretic limb.

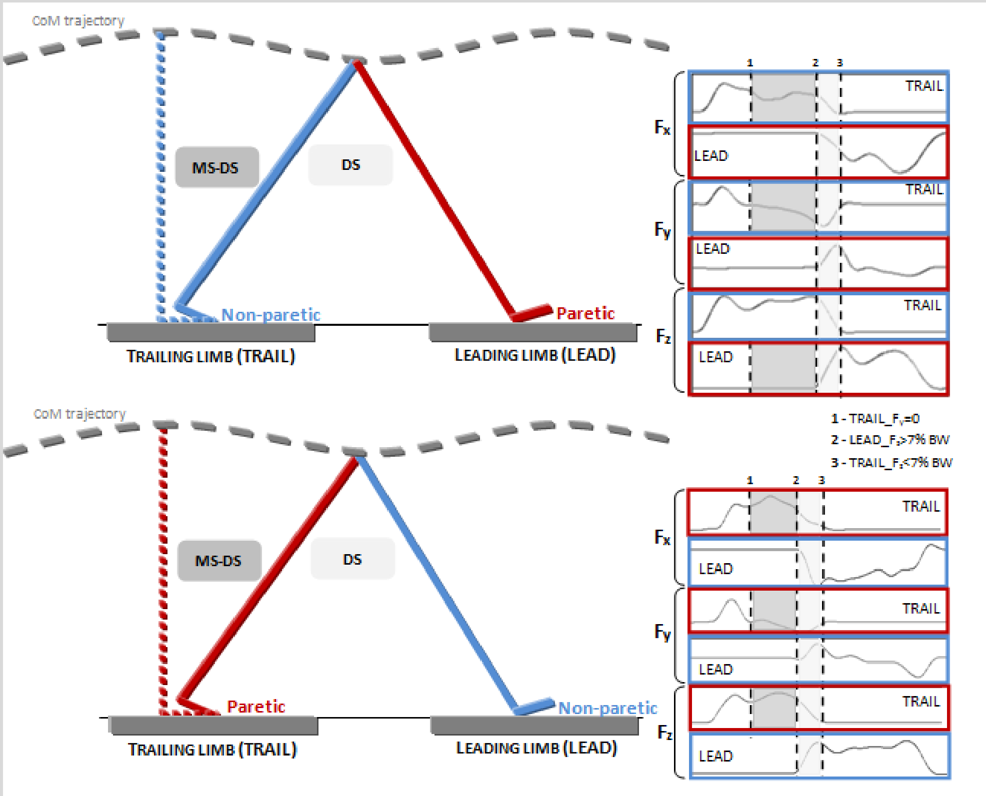


Figure 4: Representation of the intervals used to assess interlimb relation during the stance phase of walking in stroke subjects. The intervals were defined using the ground reaction force signal and the same criteria used in *Article IV*.

The results obtained, which are described and discussed in *Article V,* suggest that neuro-motor problems in stroke subjects affect both sides, being the alterations found in the paretic limb the combination of both contra-lateral and ipsilateral disposal systems dysfunctions. Lesion at the ipsilateral systems that influence directly postural control at the non-paretic side may justify part of the problems observed in the paretic limb in walking.

It should be noted that the findings obtained can be extended only to subjects with stroke lesions affecting cortico-reticular neuronal pathways, most likely to occur in subcortical levels in the territory of the middle cerebral artery (Drew, et al., 2004). Reticulospinal pathway is involved in postural control and exerts a strong influence over interneurons that mediate information by group II afferences of both lower limbs during walking (Davies & Edgley, 1994; Jankowska, et al., 2003; Matsuyama, et al., 2004). The importance of this system is highlighted during the moment of touchdown because at that point one has to match both legs muscle activity with the ground surface (Sousa, Silva, et al., 2012b). This happens during step-to-step transition where the CoM is inside the base of support (Winter, 1995) and the limbs act to maintain the CoM velocity (Donelan, et al., 2002a; Kuo, et al., 2005, 2007) and stability (Winter & Eng, 1995; Yang, et al., 1990). For this reason, coordination between posture and movement involving the dynamic control of the CoM in the base of support (Stapley et al., 1999) is important to maintain postural stability and movement efficiency (Shumway-Cook & Woolacott, 2000, 2007).

4.3 Postural control reorganisation

In the past, unstable support base conditions (see-saw) were used as a way to complicate balance maintenance and to study local oscillations of body parts (Dietz & Berger, 1982; Dietz, et al., 1980). However, support stability variation might provide an important instrument for investigating the role of muscle proprioception, as well as visual and vestibular information in the control of human vertical posture and postural control reorganisation strategies (Dietz, et al., 1992; Fitzpatrick, et al., 1994; Gurfinkel, et al., 1995; Ivanenko et al., 1997; Krizková et al., 1993). During quiet stance on a flat, stable platform individuals sway slightly and the body oscillates around the ankle-joint axis like an inverted pendulum (Winter, et al., 1998). When standing on a see-saw, humans project the CoM onto the see-saw´s point of contact with the floor (Ivanenko, et al., 1997; Ivanenko, et al., 1999). To assess the effect of this variable, we have selected a commercial shoe (Masai Barefoot Technology (MBT) shoes) that has the particularity of having a rounded sole in the anteroposterior direction, thus providing an unstable base with features common to the see-saws used in previous studies (Almeida et al., 2006; Dietz & Berger, 1982; Dietz, et al., 1980; Ivanenko, et al., 1997; Ivanenko, et al., 1999). The main advantage of selecting this kind of shoe is that subjects could wear it during daily activities, being prolongedly exposed to an unstable support base (Landry, et al., 2010; Nigg, Hintzen, et al., 2006; Ramstrand et al., 2010; Turbanski, et al., 2011). The study of postural control reorganisation in response to changes in support conditions was implemented in standing-related activity to allow the assessment of postural control mechanisms without influence from the CoM forward progression task.

The first study was carried out to assess the influence of standing in an altered support on muscle activation magnitude and its possible impact on venous return. Changes in support conditions leading for higher demands over the postural control system can impact the haemodynamic function. This influence would give important insights not only from a clinical point of view but also from an ergonomic standpoint, given the importance of applying preventive measures in this area. Apart from consequences for the locomotor system, the prolonged standing posture, requiring a static contraction of muscles, particularly those of the back and the legs, results in a diminished function of the calf muscles also known as "the peripheral heart" (Krijnen et al., 1998). This has been proved to be a risk factor to venous insufficiency (Hobson, 1997; Krijnen et al., 1997; Tomei et al., 1999). Taking into account this and that the amount of blood that can be ejected by the skeletal muscle pump is related to the calf mass (Smith et al., 1994), endurance (van Uden et al., 2005), type of contraction (Laughlin & Schrage, 1999) and functional efficiency (Smith, et al., 1994), it was hypothesised that the permanence in an unstable support base using unstable shoes would lead to positive effects over the venous return function. This hypothesis was formulated considering that the kind of shoes selected for this project have been proved to be associated with improved static and dynamic balance (Landry, et al., 2010; Nigg, Emery, et al., 2006; Ramstrand, et al., 2010; Turbanski, et al., 2011) and with changes in the ankle joint muscle activity (Boyer & Andriacchi, 2009; Landry, et al., 2010; Romkes et al., 2006). This work is described in (Sousa, Tavares, Macedo, et al., 2012) – *Article VI* (Part B).

Title: Influence of wearing an unstable shoe on thigh and leg muscle activity and venous response in upright standing

Authors: Andreia S. P. Sousa, João Manuel R. S. Tavares, Rui Macedo, Albano Manuel Rodrigues, Rubim Santos

Journal: Applied Ergonomics (2012), 43(5):933-939

Brief description: The purpose of this study was to quantify the effect of unstable shoe wearing on muscle activity and haemodynamic response during standing. To assess this purpose, thirty volunteers with a work requiring prolonged upright standing were divided into two groups: the experimental group (n=14) wore an unstable shoe for eight weeks, while the control group (n=16) used a conventional shoe for the same period. Muscle activity of the GM, TA, RF and BF and venous circulation were assessed in quiet standing with the unstable shoe and barefoot. Measurements were carried out at two moments, one before wearing the unstable shoe and the other after the eight-week period. In the first measurement the results demonstrated that there was an increase in GM activity in all volunteers while wearing the unstable shoe. On the other hand, after wearing the unstable shoe for eight weeks these differences were not verified. Venous return increased in subjects wearing the unstable shoe before and after training. These results demonstrate that the unstable shoe produced changes in electromyographic characteristics that favoured venous circulation even after training accommodation by the neuromuscular system.

The results presented and discussed in (Sousa, Tavares, Macedo, et al., 2012) indicate that standing in altered support base leads to changes in muscle activity levels at the ankle joint, suggesting a higher demand over the postural control system. These results lead to the need of understanding how the nervous system adapts in response to altered support conditions. To characterise postural control in this condition, measures of postural performance, effectiveness and of global activity were investigated (Collins & De Luca, 1993; Maurer & Peterka, 2005; Pavol, 2005; Rocchi et al., 2004). Also, muscular and postural synergies were assessed to evaluate the strategy used by the CNS, reflected by the weighted combination of postural control commands: one organised for reciprocal activation of antagonist muscles and another for their co-activation (Feldman, 1980a). The synergy between agonist and antagonist muscles around a joint can determine a condition of higher stability or higher mobility (De Luca & Mambrito, 1987; Levin & Dimov, 1997; Osternig et al., 1995; Yamazaki et al., 1994) and is modulated according to task requirements (Levin & Dimov, 1997). This study is described in (Sousa, Macedo, Santos, Silva, et al., 2012) – *Article VII* (Part B).

Title: The influence of wearing unstable shoes on upright standing postural control in prolonged standing workers

Authors: Andreia S. P. Sousa, Rui Macedo, Rubim Santos, Filipa Sousa, Andreia Silva, João Manuel R. S. Tavares

Journal: Submitted to an International Journal.

Brief description: The purpose of this study was to study the influence of prolonged wearing of unstable shoes on standing postural control in prolonged standing workers. To achieve this purpose, the same sample used in the previous study (Sousa, Macedo, Santos, Silva, et al., 2012) was used. Stabilometry parameters related to the centre of pressure (CoP), rambling (RM) and trembling (TR) were assessed and total agonist and antagonist muscle activity, co-contraction index (CCI) and reciprocal activation (R) were calculated using electromyography before and after the eight-week period. The results demonstrated that wearing unstable shoes led to: (1) decreased root mean square (RMS) and displacement and increased mean velocity (MV) of RM, (2) increased TR displacement and decreased TR RMS. No differences were observed in CoP related values and in the synergy between agonist and antagonist muscles. These findings demonstrate that wearing the unstable shoe led to increased effectiveness and performance of supraspinal processes and of spinal reflexes and/or in the intrinsic mechanical properties of muscles and joints, and that the instability provided by wearing unstable shoe in standing postural control remains even after adaptation by the postural control system.

Although quiet standing research allows studying certain aspects of postural control, it presents some limitations in revealing balance mechanisms and as a diagnostic tool to pinpoint deficits of the system (Winter, 1995). In fact, postural adjustment cannot be deduced from quiet standing posture alone (Moya et al., 2009). As a consequence, the study of the influence of an altered support condition in upright postural control was followed by studies including conditions of higher postural challenge. The study of the immediate influence of altered support on postural adjustments in a condition of high postural challenge is described in (Sousa, Macedo, Santos, & Tavares, 2012) – *Article VIII* (Part B).

Title: The influence of wearing unstable shoes on compensatory control of posture

Authors: Andreia S. P. Sousa, Rui Macedo, Rubim Santos, João Manuel R. S. Tavares

Journal: Submitted to an International Journal.

Brief description: This study investigated the influence of wearing unstable shoes on compensatory postural adjustments associated to an external perturbation. To attain this purpose, the same sample studied in (Sousa, Macedo, Santos, Silva, et al., 2012) was used. Subjects stood on a force plate resisting an anterior-posterior horizontal force applied to a pelvic belt via a cable, which was suddenly released, under two conditions: barefoot and wearing unstable shoes. The electromyographic activity of GM, TA, RF, BF, RA, and ES muscles and the CoP displacement were acquired to study compensatory postural adjustments, Figure 5. The electromyographic activity was used to assess individual muscle activity and latency, co-contraction index (1) and reciprocal activation (2) at joint and muscle group levels:

(1) $$CCI = \frac{Antagonist}{Agonist + Antagonist} \times 100,$$

(2) $$R = Agonist - Antagonist,$$

The time window selected to study all these variables was based on the neurophysiological mechanisms related to postural control, Figure 6. The results indicate that, compared to barefoot, the wearing of unstable shoes led to: 1) increased electromyographic activity of the GM, 2) increased total agonist activity, 3) decreased co-contraction index at the ankle joint and muscle group levels, 4) an increase of reciprocal activation at the ankle joint and muscle group levels, and 5) a decrease of all muscle latencies. No differences were observed in the CoP displacement between conditions. These results demonstrate that wearing unstable shoes leads to changes in neuro-muscular mechanisms that are responsible for controlling and monitoring the postural sway during both standing and in response to an external perturbation without compromising postural control performance.

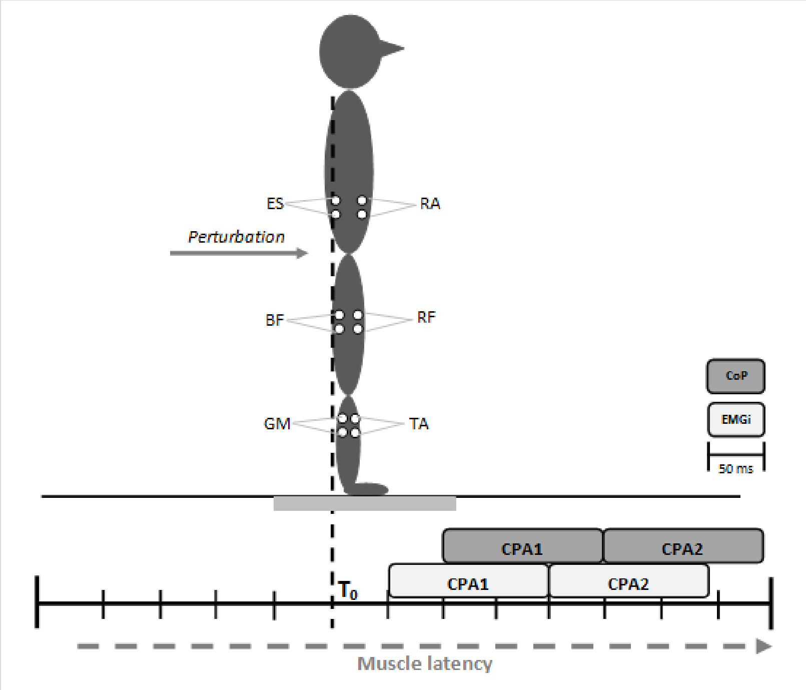


Figure 5: Schematic representation of the protocol used to assess postural control in response to an external perturbation while standing.

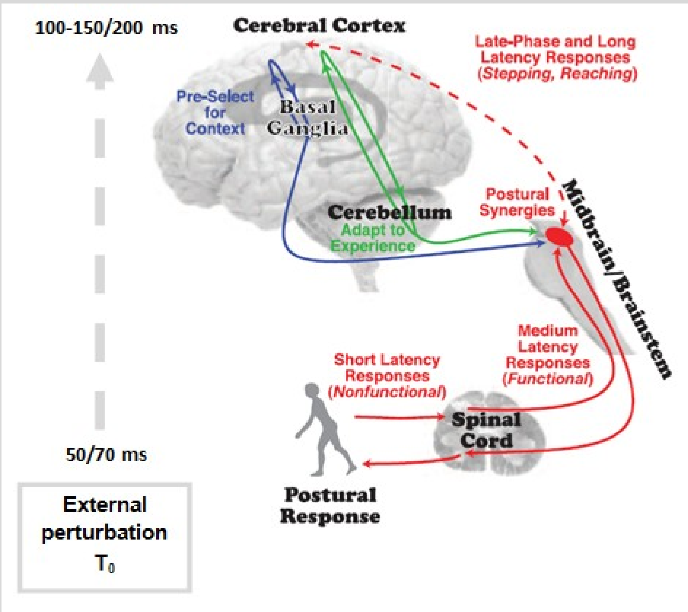


Figure 6: Mechanism involved in automatic postural control responses to an external perturbation (adapted from (Jacobs & Horak, 2007)).

One of the most successful models in motor control is based on the idea that we continuously compare our internal model of movement with the one which is produced, using the error signal to adjust motor output (Wolpert et al., 1998). During continuous perturbations using a repeated profile of support surface movement, perturbations become predictable and the postural challenge, although still balance threatening, becomes more manageable as the balance control system is set to minimise these effects (Corna et al., 1999; Hlavacka et al., 1992; Meadows & Williams, 2009; Perrin et al., 1997; Vidal, 1982). The study of the long-term influence of altered support on postural adjustments in a situation of high postural challenge is addressed in (Sousa, Macedo, Silva, et al., 2012) – *Article IX* (Part B).

Title: Influence of long-term wearing of unstable shoes on compensatory control of posture: An electromyography-based analysis

Authors: Andreia S. P. Sousa, Andreia Silva, Rui Macedo, Rubim Santos, João Manuel R. S. Tavares

Journal: Submitted to an International Journal.

Brief description: This study investigated the influence of long-term wearing of unstable shoes on electromyographic activity during compensatory postural adjustments to an external perturbation. To assess this purpose the same sample studied in (Sousa, Macedo, Santos, Silva, et al., 2012) was used and the same protocol adopted in (Sousa, Macedo, Santos, & Tavares, 2012) was implemented. The results obtained indicate that long-term wearing of unstable shoes led to: (1) an increase of BF activity, (2) a decrease of GM activity, (3) an increase of co-contraction index and (4) a decrease of reciprocal activation at leg and muscle group levels in the unstable shoe condition. Additionally, wearing unstable shoes led to a decrease in the CoP displacement, although no differences were observed in the muscle onset and offset. These results suggest that the prolonged use of unstable shoes leads to higher levels of pre-synaptic inhibition at leg and muscle group levels associated to a decreased CoP displacement.

4.4 Related works

In parallel with the works previously exposed, additional related studies were performed. Despite such additional works resulted in five articles already published, only three of them related to the study of lower limb muscle activity, antagonist muscle synergies and postural adjustment during functional activities in stroke subjects, are included as they contributed to the interpretation of the results obtained as to interlimb relation during the stance phase of gait. It should be noted that the same criteria were used in all studies presented for the inclusion of stroke participants. This fact enables comparing in a valid way the findings obtained in the main works (*Articles IV-V*) and the findings obtained in the additional works.

Title: Soleus activity in post-stroke subjects: Movement sequence from standing to sitting.

Authors: Augusta Silva, Andreia S. P. Sousa, Rita Pinheiro, João Manuel R. S. Tavares, Rubim Santos, Filipa Sousa

Journal: Somatossensory and Motor Research (2012), 29(3):71-76

Brief description: The study of muscle activity of non-paretic and paretic limbs in stroke subjects in symmetric functional tasks like the beginning of the stand-to-sit movement sequence, allows assessing the capacity of each limb to modulate the activity of plantar flexors, the main agonist in upright standing, to permit the forward rotation of the tibia over the foot while preserving its antigravitic function. The optimal regulation of postural and movement control depends on the modulation of Ib afferent information to allow the adjustable tension/length relation compatible with the maintenance of antigravity postural control. The study of the beginning of stand-to-sit allows infering about the efficiency of postural control in each limb. In this sense the main purpose of this study was to analyse the behaviour of the SOL activity during the beginning of stand-to-sit sequence in stroke subjects. To assess this purpose ten post-stroke subjects and ten healthy control subjects were monitored as to the bilateral electromyographic activity of the soleus and the ground reaction force during stand-to-sit sequence. The root mean square of the electromyographic activity was calculated for the period of increase of anteroposterior component of the ground reaction force, which occurs at the time of forward translation of the tibia over the foot, Figure 7.

The results demonstrated that when compared to healthy subjects stroke subjects showed a decreased SOL activity in the non-paretic limb, indicating a higher deviation in postural control in this limb. Neurophysiologically, these findings could result from a possible lesion of cortico-reticular system or dysfunction of the reticular system that is connected with postural control and has an ipsilateral disposition.

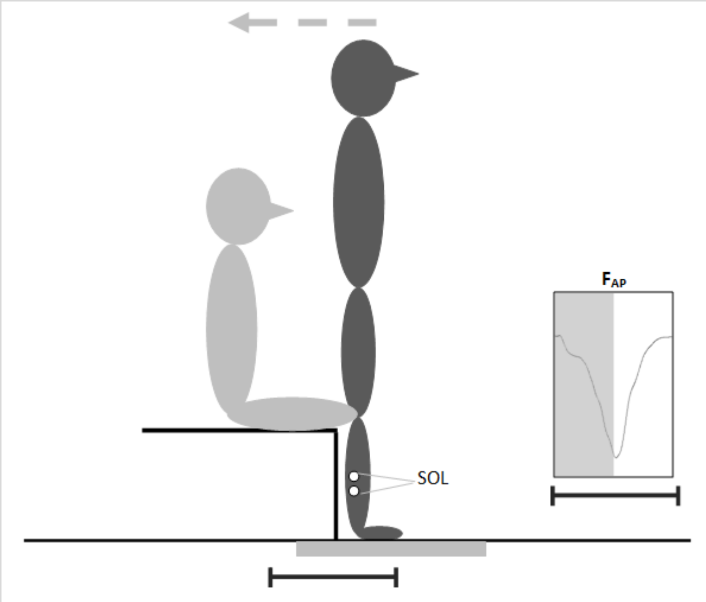


Figure 7: Representation of the interval used to calculate the root mean square of the electromyographic activity of the SOL during the stand-to-sit movement sequence.

Contribution of this work to the main purposes of the PhD project:

The conclusion obtained presents an argument for considering changes occurring in the non-paretic limb as a result of supraspinal disturb of systems more related to postural control, instead of interpreting the changes only as a result of an adaptation mechanism related to paretic limb impairment.

Title: Ankle dynamic in stroke patients: Agonist vs. antagonist muscle relations.

Authors: Augusta Silva, Andreia S. P. Sousa, João Manuel R. S. Tavares, Ana Tinoco, Rubim Santos, Filipa Sousa

Journal: Somatossensory and Motor Research (2012), 29(4):111-116

Brief description: In this study a comparative analysis was performed between non-paretic and paretic limbs in stroke subjects in terms of the magnitude and degree of antagonist muscle activation of ankle muscles. Twelve subjects with a stroke episode participated and the electromyographic signal of TA and SOL and the ground reaction force were acquired while subjects walked at their self-selected speed. Values of ground reaction force were used to divide the stance phase of gait into heel strike, midstance, and propulsion, Figure 8. In each sub-phase, the magnitude of TA and SOL was calculated, as well as the level of the antagonist coactivation.

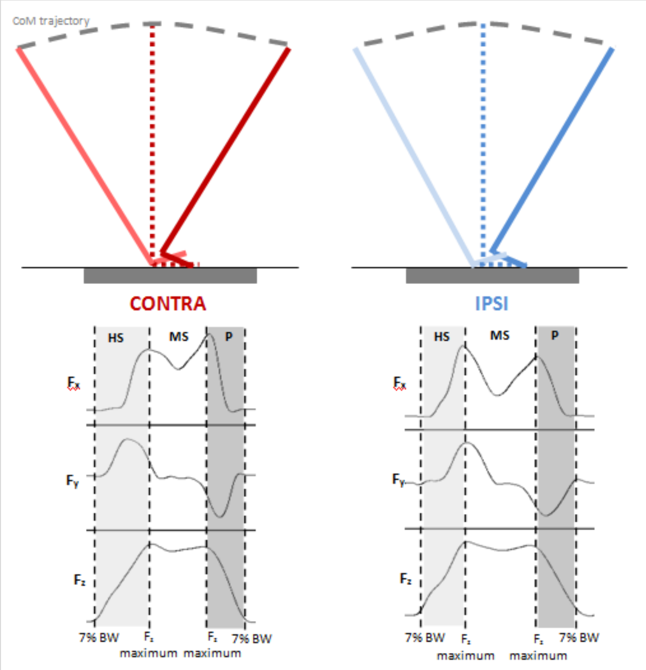


Figure 8: Representation of the protocol used to assess the antagonist coactivation during the stance phase of walking.

Although no statistical differences were found, the mean values of SOL electromyographic activity were lower in the non-paretic limb in all stance phases when compared to the paretic limb, and the opposite was observed in the TA electromyographic activity. Moreover, higher mean levels of antagonist coactivation were only found during heel-strike in the paretic limb and in the other sub-phases in the non-paretic limb. Besides, statistical differences were observed only during midstance. These results suggest that in stroke subjects the antagonist coactivation level during the midstance of gait may reflect the dysfunction of the neuronal system over the non-paretic limb.

Contribution of this work to the main purposes of the PhD project:

The conclusion obtained demonstrate that also during an asymmetric task like gait the changes occurred in the non-paretic limb can be related to a dysfunction of structures related to postural control and in the paretic limb they can be related to a dysfunction of structures associated to movement control.

Title: Activation timing of soleus and tibialis anterior muscles during sit-to-stand and stand-to-sit in post-stroke vs. healthy subjects

Authors: Augusta Silva, Andreia S. P. Sousa, Rita Pinheiro, Joana Ferraz, João Manuel R. S. Tavares, Rubim Santos, Filipa Sousa

Journal: Somatossensory and Motor Research (2013), 30(1): 48–55.

Brief description: In this study, postural control strategies, specifically anticipatory postural adjustments during sit-to-stand and stand-to-sit, were evaluated in stroke subjects. Ten healthy subjects and ten subjects with history of stroke participated. The muscle latency of SOL and TA was analysed during stand-to-sit and sit-to-stand in the non-paretic and paretic limbs in stroke subjects, and in one limb in the healthy subjects. A force plate was used to identify movement onset. In both sequences, the SOL activation timing occurred prior to movement onset in the stroke group, contrary to the pattern observed in the healthy subjects. Statistically significant differences were found in SOL activation timings between paretic and non-paretic limbs of the stroke and between these and the healthy group, but no significant differences were found between the non-paretic and the paretic limb. The TA activation timing seems to be delayed in the paretic limb when compared to the healthy subjects and showed also a better organisation of TA timing activation during stand-to-sit when compared to sit-to-stand. These results indicate that, compared to the healthy subjects, postural adjustments seem to be altered in both limbs of the post-stroke subjects, with the SOL activation timing being anticipated in both sit-to-stand and stand-to-sit.

Contribution of this work to the main purposes of the PhD project:

The conclusion obtained demonstrates changes in postural adjustments in both limbs in stroke subjects.

Globally, the additional related studies suggest that stroke subjects present changes of motor patterns in both limbs and that in both these changes can be related to neuronal damage. This perspective gave important insights into the understanding of the results obtained about interlimb relation during gait in stroke subjects.

4.5 Methodological considerations

In all experimental research studies, decisions have to be made regarding methodological parameters to assess the purposes established. As for the work carried out under this PhD thesis, some of these decisions were not fully justified in the articles included in Part B. Such decisions are mainly related to the sample selection criteria and the selection of biomechanical parameters and are detailed in the following sections.

4.5.1 Samples

Subject selection, Figure 9, was based on the specificities of each study, as well as on the global purposes of the PhD thesis. Besides anthropometric characteristics, another determinant factor in physical performance is the lifestyle adopted. Indeed, it has been demonstrated that sedentary subjects have different neuromuscular characteristics if compared to active subjects that have a direct influence on task performance (Lattier et al., 2003). Specifically, sedentary subjects have lower strength (Sööt et al., 2005), lower coordinative neuromuscular capacity (Cottini et al., 1996) and differences in contractile characteristics (Amiridis et al., 1996). These differences have been expressed in functional activities like gait as decreased gait cadence was associated with lower ankle dorsiflexion strength, and decreased stride length was associated with lower hip extension strength (Lord et al., 1996). It is important to note that in this PhD project sedentary lifestyle was viewed in the sense of low level of physical activity (Bennett et al., 2006).

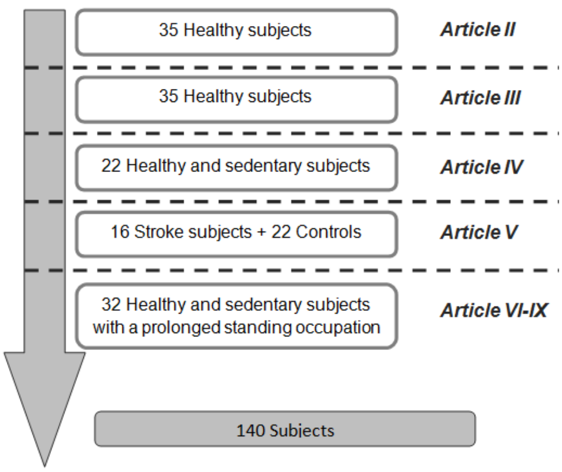


Figure 9: Representation of the “overall sample” that participated in the studies carried out during this PhD project (n=138).

Taking this into account, the criterion as to the lifestyle adopted was particularly important in *Articles IV-IX*. In *Article IV* and *Article V* the importance relied on the fact that interlimb relation was studied in healthy subjects and in stroke subjects. To assess in a valid and reliable way the impact of a lesion of neuronal structures influent on the efferent control of gait, the healthy group was composed by sedentary subjects to ensure higher homogeneity between groups as to the lifestyle adopted. Also, in *Article VI* sedentary subjects were included because they are in risk of developing peripheral venous-related pathologies (Beebe-Dimmer et al., 2005). Moreover, because standing, and specifically its duration, was recognised as a vital contributor to chronic vein insufficiency (Tomei, et al., 1999), as a result of profound hemodynamic changes (Smith, et al., 1994), the study included sedentary subjects with a standing occupation. Specifically, it included subjects spending at least 50% of the working time in a standing position (Krijnen, et al., 1998; Tomei, et al., 1999). In *Studies VII-IX*, the lifestyle criterion was also important since subjects with a sedentary lifestyle present a poor postural control (Gopalai & Senanayake, 2010). In all studies, subjects were classified as sedentary or physically active according to the time spent on physical activities, considering the criterion defined in (Pate et al., 1995). According to this criterion, subjects were classified as sedentary when their practice of physical activities was less than three times per week during 20 minutes of continuous vigorous physical activities or less than 5 times per week during 30 minutes of continuous or intermittent moderate physical activities (Ainsworth et al., 1993; Pate, et al., 1995) for at least the last 2 years (Sööt, et al., 2005).

Subjects with history of stroke were selected to study the relation between asymmetric motor impaired lower limbs during step-to-step transition. This selection was based on the asymmetric gait pattern well documented in persons who have sustained a stroke (Brandstater et al., 1983; Olney & Richards, 1996; Wall & Turnbull, 1986) and on the fact that the ability to walk gets impaired in more than 80% of post-stroke subjects (Duncan et al., 2005; Wevers et al., 2009). Subjects with lesion in the territory of the middle cerebral artery were selected because from all vascular territories affected in stroke this artery is the most frequently affected (Mohr et al., 2004). Considering the importance of the infarct location for predicting functional outcome after a stroke and that middle cerebral artery supplies either cortical and sub-cortical structures (Crafton et al., 2003; Schiemanck et al., 2006) more specific criteria had to be considered as to the location of injury. Therefore, stroke subjects with injury at the internal capsule (subcortical level) were selected given the potential impact of a lesion at internal capsule level on interlimb coordination. Indeed, the cortico-ponto-reticulospinal-spinal interneuronal system, Figure 10, has many characteristics that suggest both a potential role in modulating interlimb coordination in each locomotor cycle and in producing coordinated postural responses during locomotion (Matsuyama, et al., 2004). It should be noted that in Figure 10 the role of the cerebellum in walking regulation is not represented. Another aspect that has been considered was the time of evolution since the stroke episode. Chronic stroke subjects were selected as they present a stable neurological status (Suenkeler et al., 2002) and to enable the use of the results obtained (*Article V*) to create a rationale to target rehabilitation strategies for chronic stroke subjects. This could be significantly relevant in the rehabilitation area considering the elevated costs associated with rehabilitation of chronic stroke subjects (Gorelick, 1994) and the lack of scientific knowledge to support rehabilitation guidelines to promote interlimb relation enhancing the gait economy. The importance of studying chronic stroke subjects is also sustained by the evidence of functional improvement beyond the acute phase after stroke (6 months) (Ferrucci et al., 1993), which can be explained by neuroplasticity physiology knowledge (Hallett, 2001).

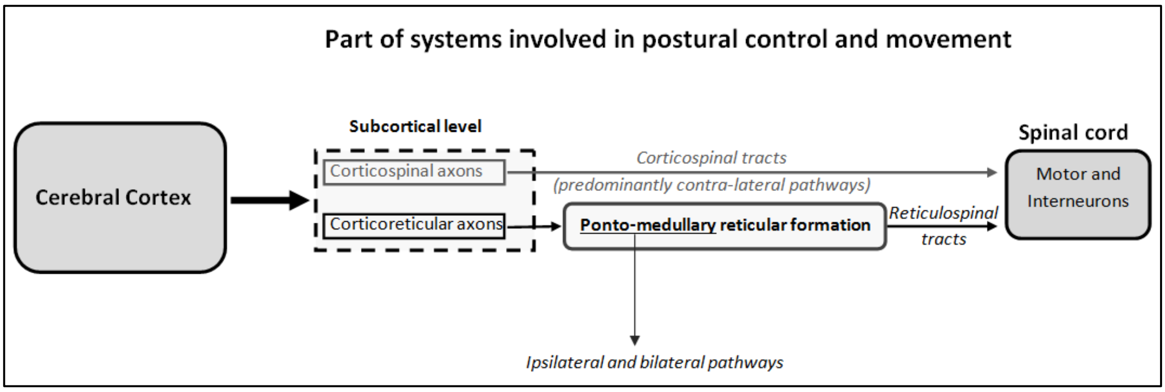


Figure 10: Representation of part of the neuronal systems involved in postural control and movement.

The corticoreticular axons originate primarily from regions of the sensorimotor cortex (mostly area 6), and descend with the corticospinal axons through the internal capsule and cerebral peduncle (subcortical level). These corticoreticular axons, some of which arise as collaterals of corticospinal axons, terminate bilaterally in the pontomedullary reticular formation from which long-descending reticulospinal axons originate. The pontine and medullary reticulospinal axons descend bilaterally but mostly ipsilaterally throughout the spinal cord to motoneurons and interneurons of spinal and is related to postural control. The corticospinal tract is responsible for movement in the contra-lateral side (Matsuyama, et al., 2004).

4.5.2 Biomechanical parameters selected

Electromyographic activity

The electromyographic signal was used to assess the activation response timing (*Article VIII and IX*) and the level of muscle activation (*Articles II-IX*). The amplitude normalisation procedure adopted in each study for the EMG signal was selected according to the review presented in the *Book chapter* included in Part B. Specifically, in *Article II* the dynamic normalisation method was selected to reduce inter-subject variability and to provide information on the pattern of muscle activation during walking at each velocity (Winter & Yack, 1987b; Yang & Winter, 1984), as the purpose was to evaluate the influence of gait velocity on the muscle relative activity patterns along the stance phase of walking, as well as in each stance subphase. From the dynamic methods, the peak dynamic method was selected to assess the periods at which muscles were more active (Benoit et al., 2003), since the muscles selected for the study present a phasic activation along the stance phase of gait (Liu et al., 2006). However, because this method leads to values that do not indicate muscle ability to activate, it could lead to misunderstanding of the results related to the effect of gait speed on the magnitude of electromyographic activity. Taking this possibility into account, we also collected the electromyographic activity during maximal isometric contraction and performed the same analysis with the maximal isometric contraction normalisation method. The results obtained demonstrated that variations obtained with the peak dynamic method expressed the same differences in muscle activity than the method that better expresses variations in the neural drive (Allison et al., 1993; Lehman & McGill, 1999; Yang & Winter, 1984). In *Article III*, the maximum voluntary contraction normalisation method was selected to assess the relative activity of the GM muscle during propulsion. This choice was adopted for two main reasons. First, since this study has the purpose of finding a possible relation between a kinetic variable of one limb and an electromyographic parameter of GM of the contra-lateral limb, we have considered the percentage of the electromyographic activity obtained in a standardised maximal isometric contraction to express an increase or decrease in the neural drive (Allison, et al., 1993; Lehman & McGill, 1999; Yang & Winter, 1984). Also, the RMS was selected to assess the electromyographic signal power detected during voluntary contraction (Medved, 2001). Second, despite the limitations exposed in the *Book chapter* review, best values of reproducibility were found for the electromyographic activity of the GM muscle during gait using the maximal isometric normalisation method than using dynamic methods (Knutson et al., 1994). In *Article IV*, to assess interlimb relation in terms of electromyographic activity, we have selected the method that most reduced intersubject variability (mean dynamic method) (Burden et al., 2003) to the detriment of methods expressing better true changes in the activation level (maximum isometric method and peak dynamic method) (Benoit, et al., 2003). The mean dynamic method represents an average of both quiet and active periods of the gait cycle (Benoit, et al., 2003) and as a result would express a possible interlimb relation between the active muscles, the quiet muscles or between the active muscles of one limb and the quiet muscles of the contra-lateral limb. This kind of normalisation method was selected also according to the population studied in *Article V*, considering the inability of performing maximal isometric contraction in the stroke group. The selection of the same normalisation procedure in *Articles IV* and *V* allows comparing the results of the interlimb relation obtained in healthy and stroke subjects, respectively. As to the specific dynamic normalisation method used, the mean normalisation method was also selected according to intersubject variability (Burden, et al., 2003) having in mind the higher variability of muscle activity pattern during walking in stroke subjects (Chau et al., 2005; Hwang et al., 2003; Kim & Eng, 2003). However, it should be noted that this method presents limitations in comparing the relative values of each muscle between stroke and healthy subjects. Although in *Article V* this method is used for comparing stroke and healthy subjects to provide a better understanding of the results obtained as to the interlimb relation, it should be noted that the differences obtained do not reflect directly muscle activation capacity and that in this study we could not validate the results obtained with an alternative method expressing the degree of muscle activation like we did in *Article II*. One possibility would be comparing the non-normalised values obtained during the stance phase of walking between groups, as performed in recent studies (Raja et al., 2012). However, considering the several factors that influence the electromyographic activity absolute value (*Book chapter*) the mean dynamic normalisation method was adopted. In studies related to stance-associated activities (*Articles VI-IX*) the maximum isometric normalisation method was adopted, as the main purpose of these studies was to analyse the effect of long-term wearing of unstable shoes on the muscle activity magnitude, and on parameters calculated from this variable to the detriment of relative patterns of muscle activity. This method allows assessing the activity level of the muscle being studied during the task as to the maximal neural activation capacity of the muscle (Allison, et al., 1993; Medved, 2001; Yang & Winter, 1984) and consequently, allows the comparison between tasks and groups.

Kinetic variables

To understand the metabolic energy cost of walking, total muscle work and force production should be analysed. However, no techniques or models are currently available that allow a valid quantification of muscle energy or muscle work in vivo. As an alternative, external mechanical work, i.e. the mechanical work performed on the CoM, can be used as a global measure of muscle work (Cavagna, et al., 1976; Willems et al., 1995). This more global measure has the disadvantage of ignoring energy flows within the body, due to movement of body segments as to the body CoM, and co-contraction around the joints (Willems, et al., 1995) that depends critically on muscle force production (Neptune, Kautz, et al., 2004; Zajac et al., 2002, 2003). However, it has the advantage of linking energy flow directly to comprehensible mechanical features of gait (Kuo, et al., 2007). During the single-support phase, where energy is generated and exchanged between stance and swing legs, a relation between muscle work and external work is not warranted (Doets et al., 2009), but during double support the external work satisfactorily estimates the total mechanical work performed on the body since the angular displacements of limbs as to the CoM are relatively small, indicating that there is little internal work (Donelan, et al., 2002b). Taking this into account, the external mechanical work was selected in *Article IV* to estimate the work during the double support phase of walking. However, external mechanical work is a resultant quantity and consequently does not include the simultaneous production of positive and negative muscle work (Neptune et al., 2001; Neptune, Kautz, et al., 2004; Neptune, Zajac, et al., 2004; Zajac, et al., 2003). This limitation is particularly relevant during the assessment of double support as one limb performs positive work (more than 97% of the double support positive work) while the other performs negative work (more than 94% of the double support negative work) (Donelan, et al., 2002b). To guarantee that external mechanical work was not substantially underestimated, the individual limbs method proposed by (Donelan, et al., 2002b) was selected for *Article IV*, since this method provides the representation of energy transfer from one limb to the other. Finally, the elastic energy stored and released by muscles is not taken into account by the external power analysis (Hof, 1998; Hof, et al., 2002; Neptune, et al., 2001). However, according to (Winter & Eng, 1995), in walking the contribution of this mechanism is quite small.

Mechanical energy analysis is a kinetic variable that enables researchers monitoring how the CNS takes advantage of energy conserving mechanisms to achieve increased movement efficiency. The major time-varying variable that can be tracked is mechanical power, which expresses energy variation rate in J/s or W (Winter & Eng, 1995) and is highly correlated with metabolic energy consumption/kg (Burdett et al., 1983). In fact, it was demonstrated that when comparing external mechanical power, internal mechanical power and joint power the first is the one presenting the highest correlation with metabolic cost during walking (Burdett, et al., 1983). As a result CoM external mechanical power and metabolic power were analysed in the study described in *Article IV*.

Mechanical power has been used to identify impairments associated with neurological deficits resultant from stroke (Olney, et al., 1991; Parvataneni et al., 2007). It has been demonstrated that about 40% of the positive work required for walking is performed by the muscles of the affected side, and the major contributors are ankle plantar flexors, hip flexors and hip extensors (Olney, et al., 1991). However, the method used to calculate the mechanical work that is based on intersegment joint powers is not sensitive to stroke severity. As a result of this limitation, the ground reaction force impulse has been used to measure the contribution of the paretic leg during walking (Balasubramanian et al., 2007; Bowden et al., 2006; Parvataneni, et al., 2007; Raja, et al., 2012), as it provides a quantitative measure of the coordinated output of the paretic leg that is sensitive to hemiparetic severity (Bowden, et al., 2006). Taking into account that assumptions for external mechanical work validity as a global measure of muscle work for the double support phase presume small displacements of the limbs as to the CoM (Donelan, et al., 2002b), which would not be guaranteed in stroke subjects, the kinetic variable used in the study described in *Article V* to assess the interlimb relation was the propulsive impulse. It should be noted that the ground reaction force impulse was selected to assess interlimb relation in stroke subjects not to represent external mechanical power but because it is sensitive to hemiparetic severity, as already stated.

Kinematic variables

Postural control in standing was assessed using stabilometry[4] (*Article VII)* as it has been demonstrated to be a reliable tool for investigating general postural stability and balance performance under specific conditions (Ruhe et al., 2010). Despite the large number of parameters that can be extracted from stabilometry, such as the dominant direction of the CoP, area covered by the CoP, and mean amplitude, range, standard deviation, velocity and frequency spectrum of the CoP (Chiari et al., 2002; Laughton et al., 2003; Maki & Ostrovski, 1993; Winter, et al., 1990), differences exist between parameters as to the most significant measure to distinguish different groups and conditions. It has been argued that time domain variables, i.e., measures of sway amplitude and velocity, may be sufficient to characterise postural sway (Collins & De Luca, 1993; Maurer & Peterka, 2005; Pavol, 2005; Rocchi, et al., 2004). Specifically, better performance has been shown by shorter trajectories or narrower areas of the CoP (Bennell & Goldie, 1994; Kinzey et al., 1997; Norris et al., 2005). While the CoP area is more immune to biomechanical factors (Chiari, et al., 2002; Rocchi, et al., 2004), the anteroposterior and mediolateral parameters can be more indicative of the true directional component of the sway (Rocchi, et al., 2004). Depending on the cause of the postural instability, velocity-related sway measures were often reported to separate stable postural control from reduced stability better than displacement-related sway measures (Horak et al., 2002; Maurer et al., 2003; Prieto et al., 1996; Rocchi et al., 2002). The CoP mean velocity has also been described as a good index of the amount of activity required to maintain stability (Geurts et al., 1993) and as the most reliable traditional CoP parameter (Ruhe, et al., 2010). The RMS has been related to postural control system effectiveness (Prieto, et al., 1996) and it was demonstrated to be sensitive to postural control perturbations (Rocchi, et al., 2002; Yamamoto et al., 1983). Considering the aforementioned, to express different dimensions of postural control in the work described in *Article VII* parameters of CoP displacements in the horizontal plane (2D) and displacements along the two orthogonal axes, as well as the values of CoP RMS and velocity, were used.

Postural steadiness is often characterised using measures based on the CoP displacement, as they reflect body segments' orientation and body movements of the CoM over the base of support (Prieto, et al., 1996), and are proportional to the ankle torque, to the combination of descending motor commands, as well as to the surrounding muscles'

[4] Measurement of forces exerted against the ground from a force plate during quiet stance.

mechanical properties (Baratto et al., 2002). However, CoP measures only represent the control variable acting to compensate CoM displacement (the controlled variable) (Morasso, et al., 1999). The importance of CoM measurements in association to CoP measurements is that the difference between the two variables is proportional to the horizontal acceleration of the CoM representing the "error" signal in the balance control system (Winter, 1995). While theoretically the CoP completely matches the CoM at low sway frequencies below 1 Hz (Winter, 1995), its displacement during sway always exceeds that of the CoM (Lafond et al., 2004). Given the importance of assessing the CoP and CoM interrelation in the study of postural control, different techniques have been proposed to assess CoM position from CoP data during quiet standing (Benda et al., 1994; Blaszczyk, 2008; Caron et al., 1997; Kuczyński, 1999; Lafond, et al., 2004; Zatsiorsky & King, 1997). The method proposed by (Zatsiorsky & Duarte, 1999, 2000) was used in the work described in *Article VII* as it provides in a reliable way the relation between CoP and CoM variables (Lafond, et al., 2004). Additionally, it has the advantage of assessing changes related to the supraspinal process, as well as to the action of spinal reflexes and changes in the intrinsic mechanical properties of muscles and joints (Zatsiorsky & Duarte, 1999). On top of that this method has been demonstrated to be sensitive to changes in ankle joint mobility during standing (Freitas, et al., 2009; Mochizuki et al., 2006). In fact, this method has been used in recent studies to assess the influence of several factors on human postural control (Danna-Dos-Santos et al., 2008; Freitas, et al., 2009; Krishnamoorthy et al., 2005; Mochizuki, et al., 2006; Rodrigues et al., 2010).

5. DISCUSSION

During this twofold-objective PhD project two important domains of motor control were assessed: movement organisation and postural control reorganisation. As to movement organisation, the purpose was to study the interlimb relation in dynamic and asymmetric tasks when both lower limbs have a supportive role (Dietz & Berger, 1984), i.e., when they are connected to a "postural program" (Horak & Nashner, 1986) and when both lower limbs have an important role in the movement (Donelan, et al., 2002a, 2002b; Kuo, et al., 2005). For this, the stance phase of walking was selected in the studies described in *Articles III-V*. This interlimb relation was studied in healthy subjects and in subjects with supraspinal damage. The second purpose, regarding postural control reorganization, was to investigate the influence of prolonged exposure to a changed afferent input related to support base stability during daily activities in postural control mechanisms. For this, functional tasks were selected to reduce the voluntary movements and in this way reliably assess the effect of changes in support conditions on the postural control pattern. In this sense, the effect of an unstable support base was studied during upright standing with (*Articles VIII and IX* and without (*Articles VI and VII*) external perturbations.

5.1 Movement organisation during the stance phase of gait

The results obtained during this project demonstrate a consistent interlimb relation during step-to-step transition (*Articles III and IV*) in healthy subjects and an impaired interlimb relation in stroke subjects (*Article V*).

From a biomechanical point of view, the results presented and discussed in *Article III* revealing that in healthy subjects the gastrocnemius activity magnitude during propulsion is related to the contra-lateral magnitude of vertical and anteroposterior components of the ground reaction force during heel strike, are perfectly justifiable considering that: 1) the energy lost during heel strike must be replaced by the contra-lateral limb (Donelan, et al., 2002a, 2002b; Kuo, et al., 2005), 2) the gastrocnemius muscle is one of the major muscles acting on propulsion (Liu, et al., 2006; Neptune, Kautz, et al., 2004), and 3) ground reaction force affects the acceleration of the CoM (Winter, 1990). It is important to note that the electromyographic activity of the gastrocnemius muscle was analysed in an interval predominantly positioned in single stance, where this muscle has the most important role (Liu, et al., 2006; Zajac, et al., 2003), while the ground reaction force magnitude was measured at an instant close to the transition between double support and single-stance. This demonstrates that interlimb relation occurs beyond the double support phase, contradicting models that have focused the study of step-to-step transition during double support (Donelan, et al., 2002a; Neptune, Zajac, et al., 2004). In fact, it has been argued that the energy loss can be reduced through the application of a propulsion impulse in the trailing limb immediately before collision of the leading limb (Kuo, 2002; Kuo, et al., 2007). This out-of-phase interlimb relation is perfectly justifiable from a postural control perspective also, as the onset of anticipatory postural adjustments may be time-locked with certain events within the locomotor cycle rather than the onset of the prime mover (Nashner & Forssberg, 1986). In this sense, from a neurophysiological perspective, it can be stated that the gastrocnemius muscle activity magnitude during unipodal propulsion would be partly explained as a feedforward mechanism in relation to the collision of the leading limb to the ground as a result of proprioceptive information provided by the leading limb. The proprioceptive information associated with this interlimb relation cannot be deduced from the findings obtained during this PhD thesis. However, the contribution provided by plantar cutaneous receptors seems to be relevant, as they were demonstrated to be related to parameters of ground reaction force (Kavounoudias et al., 1998; Zehr et al., 1997), to assist placing and weight acceptance at the beginning of stance (Zehr, et al., 1997; Zehr & Stein, 1999), and have an important role in balance maintenance during walking (Van Wezel et al., 1997; Zehr, et al., 1997; Zehr & Stein, 1999). An interlimb muscle synergy between trailing unipodal propulsion and leading limb during double support was also observed in the study described in *Article IV*. It has been verified that the activity of the main responsible for impact reduction during heel strike (Neptune, Zajac, et al., 2004; Sadeghi et al., 2002; Whitle, 2007; Zajac, et al., 2003) was inversely related to the activity of the same muscles and positively related to the SOL activity during contra-lateral unipodal propulsion. In fact, the results obtained in studies described in *Articles III* and *IV* demonstrate a reciprocal influence between the leading limb during double support and the trailing limb during unilateral propulsion, as proprioceptive information related to ground reaction force can be used to create feedforward commands to regulate contra-lateral plantar flexor activity in the preceding subphase of walking, but also because the main responsible for impact reduction during heel strike are influenced by the homologous activity and by the SOL of the contra-lateral limb during unipodal propulsion.

Based on results presented in *Article III* and on the role of SOL and RF muscles in forward propulsion during double support (Neptune, Kautz, et al., 2004), a relation between these muscles of the trailing limb and the ground reaction force of the leading limb during double support can be expected. However, our results do not allow making assumptions as to this aspect. The results discussed in *Article IV* revealed an inverse relation between the main responsible for impact reduction during leading heel strike and the main agonists during pre-swing (Winter, 2005). This interlimb relation could be partly mediated by low-threshold group Ia, Ib, or II afferents, or a combination of some or all of these at spinal level (Stubbs & Mrachacz-Kersting, 2009). From a biomechanical point of view, the results obtained suggest that despite being responsible for the leg energy absorption and consequent negative work (Winter, 2005), higher activity of VM, BF and

TA, reducing the leading limb heel strike impact (Sadeghi, et al., 2002; Whitle, 2007; Zajac, et al., 2003), leads to a lower need of muscle activity of plantar flexors, the main responsible for positive work of the trailing limb (Winter, 2005). This interpretation is in accordance with the biomechanical models proposed for step-to-step transition (Kuo, et al., 2007) since the energy dissipated is related to an inelastic collision of the swing leg with the ground, leading to changes in velocities of the legs and the CoM that need to be compensated by active work by the trailing limb. The importance of plantar flexors' role in restoring energy loss during step-to-step transition is highlighted by the higher magnitude of positive mechanical work as to the magnitude of the negative work (*Article IV*), which reflects the results obtained previously as to total positive and negative muscle work in walking (DeVita et al., 2007). The results obtained in the work described in *Article IV* demonstrate clearly that positive work of the trailing limb depends strongly on negative work performed by the leading limb. The interlimb relation observed is mainly related to forward progression and the importance of that is expressed by the impact of anteroposterior components of the trailing limb positive mechanical power and of the leading limb negative mechanical power over the metabolic cost of walking (*Article IV*).

While interlimb relation between unipodal propulsion and heel strike is perfectly integrated in healthy subjects, the findings presented in *Article V* demonstrated that in stroke subjects only the activity of the paretic limb is influenced by the activity of the non-paretic limb, indicating that paretic limb motor pattern generation may be substantially influenced by contra-lesional neural activity related to the non-paretic leg sensorimotor state during bilateral lower limb function. In association with the damage of important areas for interlimb relation, consistent evidence exists about the disruption of group II pathways (Marque, et al., 2001; Maupas, et al., 2004; Nardone & Schieppati, 2005). Bearing in mind that impairment of the processing of peripheral afferent information in post-stroke subjects might contribute to abnormal muscle activity because the sensory feedback makes an important contribution to the centrally generated motor commands during human locomotion (Sinkjær, et al., 2000), the deregulation of group II pathways could explain the fact that only the paretic limb was influenced by the non-paretic limb. Based on this, more "appropriate" sensorimotor information from the non-paretic limb (the less affected) would be integrated by the nervous system and would contribute to a more appropriate paretic pattern. However, this hypothesis could be questioned considering that motor impairments on the non-paretic side have been reported in both the upper (Colebatch

& Gandevia, 1989; Desrosiers et al., 1996; Jones et al., 1989) and lower extremities (Adams et al., 1990; Lindmark & Hamrin, 1995). These motor impairments have been described to result from higher function deficits of the contra-lateral limb (Aruin, 2006), from disuse but also from neuronal damage depending on the anatomical region of vascular disruption (Matsuyama, et al., 2004). Taking into account the additional works related to this PhD thesis that were presented, it appears that non-paretic disorders related to lower SOL activity level, higher coactivation values and changes in feedforward mechanisms are related to a dysfunction of the ventral-medial system in sub-cortical injuries located at the internal capsule level (Silva, Sousa, Pinheiro, Ferraz, et al., 2012; Silva, Sousa, Pinheiro, Tavares, et al., 2012; Silva, Sousa, Tavares, et al., 2012). In fact, the results discussed in *Article V* demonstrate that the non-paretic limb exerts a positive influence, but also a negative influence over the paretic limb when this limb exerts a higher support role. This suggests that paretic performance in double support is not only explained by the neuronal damage but also by the influence of the non-paretic limb. Based on related work to our main investigation (Silva, Sousa, Pinheiro, Ferraz, et al., 2012; Silva, Sousa, Pinheiro, Tavares, et al., 2012; Silva, Sousa, Tavares, et al., 2012) and on the results presented in *Article V,* it can be argued that the negative influence of the non-paretic limb over the paretic limb can be attributed to changes in postural control over the non-paretic limb. This possibility assumes significant relevance in double support considering the higher demand imposed over the postural control system (Falconer & Winter, 1985; Lamontagne, et al., 2000; Onley, 1985). These findings support the argumentation for the dysfunction of the ventral-medial system over the non-paretic limb as one of the causes for impaired interlimb relation in stroke subjects.

Walking after stroke is characterised by slow gait speed (von Schroeder et al., 1995), poor endurance (Dean et al., 2001) and a reduced ability to adapt to the task and to environment constraints (Said et al., 1999). Motor impairments are believed to be the primary cause for this poor walking ability as suggested by the association between muscle weakness of specific muscle groups, such as the plantar flexors on the paretic side, and the slow speed (Nadeau et al., 1999; Olney, et al., 1994). Stroke subjects studied in the work described in *Article V* adopted an average walking speed that follows values presented by earlier studies (Olney & Richards, 1996) and is characteristic of limited community ambulation, corresponding to a moderate impairment (Perry et al., 1995). This low gait speed in stroke subjects is less cost effective (Hesse et al., 2001), however, it provides them with a rate of energy expenditure (Detrembleur et al., 2003) and muscle use ratios or levels of effort (Milot et al., 2007; Requião et al., 2005) that are similar to those of healthy subjects walking at a comfortable speed. Based on this, the lower speed in post-stroke subjects has been hypothesised to be an adaptive strategy for energy conservation (Lamontagne, et al., 2007). Whereas correlation analysis have revealed that some EMG abnormalities such as spasticity (Lamontagne et al., 2001), altered cocontraction (Lamontagne, et al., 2000), and muscle paresis (Lamontagne, et al., 2002) are more pronounced in subjects with low gait speed, a cause-effect relationship of some of these abnormalities with poor locomotor performance (Detrembleur, et al., 2003) remains difficult to establish. The study of the interlimb relation during the stance phase of gait in stroke subjects can give significant insights for understanding the low performance of stroke gait, considering the importance of step-to-step transition performance in global gait efficiency.

Globally, the results demonstrate that the activity of the non-paretic limb is not related to the activity of the paretic limb, which is confirmed by the results obtained as to impulse, and that the paretic limb, presenting lower contribution to forward progression, adjusts its activity according to the activity of the non-paretic limb, which is also confirmed by force impulse values. Paresis of ankle plantar flexors muscles has been demonstrated to be the main cause of the reduced plantar flexor moment of the paretic limb during the stance phase of gait (Lamontagne, et al., 2002). The results discussed in *Article V* indicate that the non-paretic limb is in part responsible for the reduced moment of plantar flexors in forward progression.

5.2 Postural control reorganisation

Postural control reorganisation in response to an unstable support base

The results presented in *Article VII* demonstrate that the kind of unstable support base used in this project (unstable shoes) leads to an increased postural sway expressed by changes in most of the representative CoP displacement parameters (Collins & De Luca, 1993; Maurer & Peterka, 2005; Pavol, 2005; Rocchi, et al., 2004), reflecting a situation of high postural demand. This higher postural sway could have two opposite interpretations. On the one hand, it could be associated to a condition of lower performance by the postural control system (Horak, et al., 2002; Maurer, et al., 2003; Prieto, et al., 1996; Rocchi, et al., 2002; Ruhe, et al., 2010). On the other hand, the higher postural control demand (*Article*

VII) associated to a higher active control at the ankle level (*Articles VI and VII*) would lead to an increased proprioceptive acuity provided by ankle muscles (Gandevia et al., 1992) as a result of a higher fusimotor drive (Gorassini, et al., 1993; Gurfinkel et al., 1992; Ribot-Ciscar et al., 2009). The results presented and discussed in *Articles VIII and IX* sustain this last interpretation since a decrease of onset timing of ankle plantar flexors in response to an external forward perturbation was observed under the condition of higher support instability. The relation between an increase of proprioceptive acuity and the higher fusimotor drive of ankle plantar flexors during standing in an unstable support base is acceptable as length signals coming from the less adaptable spindles secondaries provide the CNS with an appropriate input for detecting low-frequency displacements occurring mainly about the ankle (Gurfinkel, et al., 1995) and for assisting ankle muscle reflex responses (Schieppati, et al., 1995). This interpretation is valid if we consider the studies arguing that ankle plantar flexors are the main responsible for proprioceptive information, signaling changes in body position (Fitzpatrick, Taylor, et al., 1992; Lakie et al., 2003; Loram & Lakie, 2002a; Loram et al., 2005a; Nashner, 1976). Recently, based on the fact that during quiet standing reciprocal inhibition could be more important than autogenic stretch reflex, importance has been given to the role of un-modulated muscles crossing the joint in parallel with the active agonist (Di Giulio et al., 2009). Under this perspective, the results obtained in the studies described in *Articles VI* and *VII* would be also associated with increased acuity of proprioceptive information provided by the un-modulated muscles, since no differences were observed in antagonist muscles activity in response to the unstable support base condition. In summary, the results presented in *Articles VI-IX* suggest that the higher performance of the postural system when using unstable shoes is associated with an increased proprioceptive acuity related to higher motor drive of ankle plantar flexors spindles and/or to a consequent lower motor drive over muscle spindles of the antagonist.

Studies about postural control when standing in a see-saw have devaluated the role of the information provided by muscle spindles, arguing that the higher changes in orientation provided by this kind of support surfaces turns it difficult to use proprioceptive information about the relative positions of successive links of kinematic chain for the reconstruction of a body position internal representation (Ivanenko, et al., 1997; Ivanenko & Talis, 1995). As a consequence, the authors gave more importance to the information provided by the Golgi organ tendon (Ivanenko, et al., 1997). However, it should be noted that the format of the unstable shoe used during this PhD project is different from the see-saw used in previous studies, Figure 11, leading to lower levels of changes in segment orientation at the ankle joint, justifying the maintenance of the role of muscle spindles in this condition. The influence of the information provided by cutaneous afferents of the feet should be also questioned, considering its importance in standing postural control (Roll et al., 2002; Wright et al., 2012). However the results obtained cannot support this possibility.

Figure 11: Illustration of the kind of unstable shoe adopted in this PhD project and a schematic representation of the see-saw adopted in other studies.

In association with the exposed above, standing in an unstable support base led to increased reciprocal activation at the leg, thigh segments and muscle group levels and the opposite happened as to coactivation levels (*Article VII*). Reciprocal activation of the agonist-antagonist set is present when the subject is uncertain of the task to be performed, when a quick compensatory force contraction is perceived to be required (De Luca & Mambrito, 1987) and in joints involving joint movement (Lavoie et al., 1997). Studies using see-saws and continuous rotating platforms have indicated higher joint movement associated with the need of making see-saw rotation to place the support under the CoM (Akram et al., 2008; Almeida, et al., 2006; Ivanenko, et al., 1997; Ivanenko & Talis, 1995). However, because the degree of perturbation induced by the unstable shoe selected is lower than that applied in the studies mentioned, we were expecting to find higher co-activation levels in the unstable support base condition, as co-activation has been described as the most robust strategy to counteract perturbations (Damm & McIntyre, 2008; Humphrey & Reed, 1983; Milner, 2002; Osu & Gomi, 1999) by increasing joint stiffness (Feldman, 1980a; Joyce et al., 1969; Milner et al., 1995; Nichols & Houk, 1976; Serres & Milner, 1991). However, the results obtained in *Articles VII-VIII* indicate that wearing unstable shoes leads the postural control system to rely more on reflex feedback more than on stiffness increase to compensate for the decrease of stability, which has been demonstrated to be more efficient and accurate but also more challenging for the postural control system (Aruin & Almeida, 1997; Friedli et al., 1984; Garland et al., 1997; Hogan, 1984; Hong et al., 1994; Latash, Aruin, Neyman, et al., 1995; Massion et al., 1999). This synergy pattern selection (*Articles VII-IX*) in association with the changes occurred in the magnitude of ankle muscles activity only (*Article VI*) indicate that this kind of unstable shoe leads to instability levels that are perfectly managed by the CNS as a low perturbation (Adkin et al., 2000; Santos & Aruin, 2009). In fact, the postural performance was not modified when exposed to an external perturbation (*Articles VIII-IX*).

The higher demand over postural control in an unstable support base led to changes in the neuromusculoskeletal system, mainly at the ankle joint, leading to positive effects over the venous return (*Article VI*). This could be related to the increased activity of the gastrocnemius muscle, to the kind of contraction imposed, as well as to the increased reciprocal activation.

Postural control reorganisation in response to prolonged exposure to an unstable support base

After prolonged standing in an unstable support base, the individual muscle activity, as well as the relation between agonist and antagonist, were close to the necessary for the stable support base condition (*Articles VI, VII and IX*). However, exceptions were observed at the thigh level as reciprocal activation remained higher in the unstable condition (*Articles VII and IX*) associated to an increased BF activity in automatic and voluntary compensatory response (*Article IX*) and a decrease of GM activity (*Article IX*). These findings suggest a transfer of postural control synergy for the thigh which has been reported as more beneficial to optimise postural stability (Day et al., 1993; Horak, et al., 1990; Kuo, 1993; Runge, et al., 1999; Yang, et al., 1990). In fact, CoP parameters indicate higher performance by the postural control system in compensatory responses after prolonged wearing of unstable shoes (*Article IX*), and the relation between CoP and CoM (*Article VII*) indicates a higher performance and efficiency (Bennell & Goldie, 1994; Prieto, et al., 1996) of the supraspinal process, as well as of the action of spinal reflexes and/or of the intrinsic mechanical properties of muscles and joints (Zatsiorsky & Duarte, 1999). Having in mind the important role of the information provided by group II fibers in postural control during standing (Corna, et al., 1996; Marchand-Pauvert et al., 2005; Morasso & Schieppati, 1999; Nardone, et al., 1996; Schieppati & Nardone, 1997; Schieppati, et al., 1995) it can be hypothesised, based on findings presented in *Article IX*, that the decreased gastrocnemius activity after prolonged wearing of unstable shoes could be also related with a possible decrease of medium latency responses as these are related to group II afferences, being more influenced by the postural set and being the only that have a stabilising effect during perturbation of stance (Jacobs & Horak, 2007; Nardone, et al., 1990). The maintenance of decreased values of ankle muscle latency, even after prolonged unstable shoe wearing (*Article IX*), can be related to a remaining instability effect expressed by the higher activity of total agonist activity and higher values of CoP displacement in the unstable support base condition (*Article VII*).

Findings obtained indicate that prolonged wearing of unstable shoes leads to improved postural performance and efficiency (*Articles VII and IX*). The results also demonstrate that even after adaptation by the postural control system, the venous return was higher in the unstable support base condition than in the control condition (*Article VI*). When analysing the immediate effects of standing in an unstable support base on venous return, some hypothesis were stated to explain the venous return increased values. The results obtained after prolonged standing in an unstable support base demonstrate that even after the decrease of gastrocnemius activity magnitude and of reciprocal activation levels, venous return remained higher in the unstable support base condition, suggesting that the main factor responsible for increased venous return was related to the kind of muscle contraction of plantar flexors, since dynamic muscle contractions favour venous circulation. This hypothesis is sustained by higher CoP displacement values indicated in *Article VII* after prolonged wearing of unstable shoe and by studies on see-saws (Ivanenko, et al., 1997; Ivanenko & Talis, 1995). However, future work should be undertaken to evaluate in a reliable way the kind of muscle contraction performed by ankle plantar flexors when wearing the unstable shoes.

The impact of postural control strategies on venous return is of significant relevance in healthy subjects that are in risk of developing venous insufficiency but also in subjects with pathologies related to venous insufficiency. Although the effect of an unstable support base has been explored in healthy subjects, beneficial results would be expected in subjects with venous insufficiency, as balance training has been demonstrated to promote improved postural control performance both in healthy subjects (Balogun et al., 1992; Heitkamp et al., 2001; Hoffman & Payne, 1995; Rozzi et al., 1999) and in subjects with pathology (Mattacola & Lloyd, 1997; Rozzi, et al., 1999).

6. MAIN CONTRIBUTIONS ACHIEVED

The main contributions achieved during this PhD project can be summarised as follows.

- An integrated review of the neurophysiological and biomechanical mechanisms related to movement and postural control was conducted, which contributes to a basis of knowledge for the research in the field of motor control, as this study gathers neurophysiological and biomechanical perspectives that have been normally discussed independently.

- A review of the amplitude electromyographic normalisation methods was produced. This review provides a comprehensive and comparative analysis of the different methods used in electromyography studies. The importance of this study is related to the lack of uniformity in the selection of normalisation methods in electromyographic studies and to the lack of a reflexive knowledge about the implications of each method in the interpretation of the findings obtained. In this sense, the information offered is of significant relevance in studies using electromyography to assess muscle activity.

- The studies related to interlimb relation during walking present the interlimb synergy, in terms of muscle activity and global mechanical work related to metabolic energy expenditure, used by healthy subjects. This knowledge is useful for understanding step-to-step transition during gait from a motor control perspective and for interpreting walking impairments and inefficiency related to pathologies involving lower limbs unilateral or bilateral asymmetric impairment. These pathologies could be related to musculoskeletal and neurological disorders. While there is some evidence of interlimb relation in unilateral musculoskeletal disorders, to the best of our knowledge the interlimb relation assessment in conditions of bilateral asymmetric impairment, like stroke, was not explored in previous studies. Considering that among the neurological deficits of adult life, stroke is a prevalent disorder with life-threatening consequences, the study of this pathological condition is important to gain a better understanding of performance deficits and the potential for functional recovery, as well as to develop intervention strategies that maximise recovery. The results obtained in this PhD thesis present an important contribution to rehabilitation strategies, and the main argument is the importance of including in rehabilitation programs strategies to improve postural control in the non-paretic limb, not only to improve performance of the non-paretic limb, but also to improve performance of the paretic limb (the more affected limb) during step-to-step transition. The interpretation of the findings obtained considering neurophysiological and biomechanical perspectives enabled translating the results obtained into current clinical practice.

- The results obtained as to the influence of an unstable support base on postural control provide a rationale that could be useful in the areas of Ergonomy, Rehabilitation and Vascular Medicine. In Ergonomy the results obtained are mainly related to the use of an unstable support base to improve standing postural control and venous circulation. In the rehabilitation domain, the results provide evidence to improve proprioceptive acuity associated with long-term wearing of unstable shoes, presenting arguments for the use of unstable shoes by subjects with proprioceptive impairments. In Vascular Medicine the results obtained show the potential of using unstable shoes to increase calf pump efficacy in subjects with venous insufficiency.

- The work developed within the scope of this PhD project has been disseminated by several presentations and publications, including articles in international scientific journals and conferences and a book chapter, which confirms the merits of the contributions achieved.

7. CONCLUSION AND FUTURE WORK PERSPECTIVES

Two main purposes were defined for this project. One was related to the investigation of bilateral lower limb movement organisation during step-to-step transition in healthy subjects and in subjects with asymmetric motor impairments resulting from stroke. The other main purpose was focused on postural control reorganisation in response to an unstable support base during daily activities and its impact on systems whose action is closely related to the action of the musculoskeletal system.

As to the bilateral lower limb movement organisation during step-to-step transition during walking, the results obtained indicate the existence of a consistent and reciprocal interlimb influence in terms of: (1) muscle activity, (2) muscle activity of the trailing limb and the magnitude of the ground reaction force of the contra-lateral limb, and (3) mechanical work over the CoM. Results demonstrated also that the trailing limb adjusts its activity trough a feedforward mechanism in relation to the magnitude of the ground reaction force during heel strike and influences muscle activity during heel strike. During double support there is an influence of the main muscles responsible for impact reduction during heel strike and CoM mechanical work of the leading limb over the main responsible for forward propulsion and CoM mechanical work of the trailing limb, respectively. The interlimb relation observed is mainly related to forward progression, which has been demonstrated to be the task most associated to metabolic energy expenditure.

The interlimb relation observed in healthy subjects is perturbed in stroke subjects with lesion at the internal capsule level. The results obtained demonstrate that: (1) the paretic limb presents lower performance in the forward propulsion when compared to the non-paretic limb and the control group, and (2) the paretic limb does not influence the level of activity of the non-paretic limb during step-to-step transition. Despite exerting an indirect functional influence over the activity of plantar flexors, the non-paretic LEAD limb exerted also dysfunctional influence over the paretic TRAIL during double support. These findings suggest that the lower performance of the paretic limb in forward propulsion is not only related to the contra-lateral supraspinal damage, but also to a negative influence of the non-paretic limb.

As to the second main purpose, the results indicate that exposition to the instability provided by the kind of shoes adopted leads to positive effects over the postural control system that seem to be related to higher acuity muscle spindles not only at an immediate level but also after a long-term exposure. The changes occurred in terms of ankle muscle activity in response to an unstable support base favour venous circulation even after training adaptation by the neuromuscular system.

Although this PhD thesis points to several conclusions on the interlimb relation during gait, other functional activities like standing and gait initiation should be explored. Also, in future works focus should be given to the study of subjects with stroke in an acute/subacute stage to decrease the influence of biomechanical compensatory strategies. Also, the study of subjects with Parkinson disease would give significant insights as to the interlimb relation considering the importance of basal ganglia in the initiation, execution, and termination of motor programs. Considering the importance of proprioceptive information provided by ankle muscles in the interlimb relation, future works should be developed to study subjects with ankle instability.

The findings obtained as to the positive effects of standing in an unstable support base in postural control and venous return, in healthy subjects, encourages the study of this change of afferent input in subjects with proprioceptive impairment and in subjects with venous insufficiency.

PART B – *ARTICLE I*

Biomechanical and neurophysiological mechanisms related to postural control and efficiency of movement: a review

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ABSTRACT

Understanding postural control requires considering various mechanisms underlying a person´s ability to stand, to walk and to interact with the environment safely and efficiently. The purpose of this paper is to summarise the functional relation between biomechanical and neurophysiological perspectives related to postural control in both standing and walking based on movement efficiency. Evidence related to the biomechanical and neurophysiological mechanisms is explored as well as the role of proprioceptive input on postural and movement control.

Key words: Postural stability, proprioceptive input, compensatory postural adjustments, standing, walking, gait

1. INTRODUCTION

Postural control has been defined as the control of the body's position in space for the purposes of balance and orientation (Horak, 2006; Massion, 1998; Shumway-Cook & Woolacott, 2000, 2007). Postural orientation involves the active control of body alignment and tonus in relation to gravity, base of support, environment and internal references (Horak, 2006; Kandel et al., 2000; Lundy-Ekman, 2002; Massion, 1998; Raine et al., 2009; Winter, et al., 1990). Postural equilibrium involves the coordination of sensorimotor strategies to stabilise the body's centre of mass (CoM) during both self-initiated and externally triggered disturbances in postural stability (Horak, 2006). Postural stability has been defined as the ability to control the CoM in relation to the base of support (Shumway-Cook & Woolacott, 2000, 2007). The weight of each component, i.e., of orientation and stability, varies according to the task and the environment. Indeed, the postural control system adjusts its goal under different circumstances, such as longitudinal alignment of the whole body to maintain a steady, erect stance; remodeling of stance in preparation for a voluntary movement; shaping of the body for display purposes, as in dance; maintenance of balance, as on the gymnast's beam; or conservation of energy in a demanding task (Kandel, et al., 2000; Shumway-Cook & Woolacott, 2007).

Biomechanically, a postural control position is achieved when the CoM is within the base of support and is aligned with the centre of pressure (CoP) (Winter, 1995). Any external or internal perturbation that changes the projection of the CoM to the limits of the base of support, and the alignment between CoM and CoP may lead to postural challenge.

The ability to maintain the body's CoM within a specific boundary is dictated by the efficiency of the individual's balance mechanisms (Raine, et al., 2009) related to anticipatory postural adjustments (APA), triggered by feedforward mechanisms prior to the perturbation (Aruin & Latash, 1995b; Belen'kii et al., 1967; Li & Aruin, 2007; Massion, 1992; Schepens & Drew, 2004), as well as to compensatory postural adjustments (CPA) that are initiated by sensory feedback signals (Alexandrov et al., 2005; Park et al., 2004). The process of generation of APA is likely to be affected by expected magnitude (Aruin & Latash, 1996; Bouisset et al., 2000) and direction (Aruin & Almeida, 1997; Santos & Aruin, 2008) of the perturbation, voluntary action associated with the perturbation (Arruin, 2003; Shiratori & Aruin, 2007), postural task and body configuration (Arruin, 2003; van der Fits et al., 1998). In conditions of high instability demands, the central nervous system (CNS) may suppress APA as a protection against their possible destabilising effects (Arruin, et al., 1998). In fact, a relation between APA and CPA has been demonstrated (Santos, et al., 2009), suggesting the existence of an optimal utilization of APA in postural control. The CPA response depends not only on the APA, but also on the direction and magnitude of the perturbation, the base of support dimension (Dimitrova et al., 2004; Henry et al., 1998; Horak & Nashner, 1986; Jones et al., 2008) and on the involvement in a secondary task (Bateni et al., 2004).

The main sensory systems involved in postural control are proprioception, the vestibular system and vision, and their afferent pathways within the CNS (Day & Cole, 2002; Shumway-Cook & Woolacott, 2007). Afferent and efferent pathways involve the spinal cord, the brain stem, the cerebellum, the midbrain, and the sensorimotor cortex. All of these contribute to the development of an internal representation of body posture that is continuously updated based on multisensory feedback and is used to forward commands to control body position in space (Massion, 1994; Mergner & Rosemeier, 1998). This provides a basis for all interactions involving perception and action with respect to the external world and is likely to be partly genetically determined and partially acquired through ongoing experiential learning. It is therefore, adaptable and vulnerable, is dependent on the ongoing information that it receives (Meadows & Williams, 2009) and is related to human movement variability, allowing for adjustable functional behavior (Van Emmerik & Van Wegen, 2000).

The neural process involved on stability organisation and body orientation in space is necessary practically for all dynamic motor actions (Massion, 1998). Specifically, the control of balance during gait and while changing from one posture to another requires a complex control of a moving body CoM that is not within the base of foot support (Winter, et al., 1993). In fact, human gait is influenced by a multifactorial interaction that results from neural and mechanical organisation, including musculoskeletal dynamics, a central pattern generator (CPG), based on a genetically determined spinal circuit, and peripheral and supraspinal inputs (Arechavaleta et al., 2008; Borghese et al., 1996; Horak & Macpherson, 1996a; Mazzaro et al., 2005; McCollum et al., 1995; Segers, 2006). The CPG designs spinal networks than can generate patterns of rhythmic activity in the absence of external feedback or supraspinal control. However, these spinal networks are modulated by peripheral input and supraspinal control (Armstrong, 1986; Rossignol et al., 2006).

The present study aims to review the biomechanical and neurophysiological mechanisms related to postural control in both standing and walking based on movement efficiency. In the following sections, the neural mechanisms, the role of afferent information and biomechanical aspects will be considered to upright standing and human gait.

2. Postural and movement control

2.1 Neural mechanisms

2.1.1 Upright standing

The upright stance of the human is an unstable position (Peterka & Loughlin, 2004). Postural sway reflects noise and regulatory activity of the several control loops involved in maintenance of balance, which requires that the CoM never deviates beyond the support area. The control of the appropriate level of neuromuscular activity to produce rapid postural control strategies involves medial descending systems (Raine, et al., 2009). The role of these systems is fundamental to the organisation of postural tone appropriately according to environment demands, gravity and base of support. The vestibular system action is related to postural tone adjustments to body weight support (Matsuyama & Drew, 2000). This system plays a major role in the antigravity function (Latash, 1998; Siegel & Sapru, 2011) as it is responsible, through the lateral vestibulospinal tract, for the activation of ipsilateral extensor motor neurons and their associated gamma motor neurons (Latash, 1998; Rothwell, 1994; Siegel & Sapru, 2011). The reticular formation has an important role on APA production (Schepens & Drew, 2004) as it receives afferent input from all the sensory system and also from the pre-motor cortex and supplementary motor area (Brodal, 1981; Kiernan, 2005; Rothwell, 1994). The possible role of the cortex in postural control has been discussed and relevance has been discussed and relevance has been given to the role of pre-motor cortex influence in APA production (Massion, 1992) and to the supplementary motor area as a potential focus of control for APA generation (Jacobs et al., 2009).

2.1.2 Human gait

Appropriate mechanisms for controlling muscle tone are essential to maintain stable postural and locomotor synergies in bipedal gait performance. The dependency between postural control and movement may be justified by the connection between the cortex and the reticular formation. Actually, muscle tone and the locomotor system can be controlled, in parallel, by a combined input to the brain stem of net inhibition from the basal ganglia, and net excitation from the motor cortex (Takakusaki et al., 2004). Specifically, an important neuronal circuit that allows the coexistence of postural adjustments and execution of movement is the cortico-ponto-cerebellar pathway, which allows the connection of the cortex with the nucleus of the brain stem and cerebellum (Ito, 2006). With this circuit, the postural control can be organised ipsilateraly to the activated side with respect to the control of movement in the contra-lateral side. This relationship between movement and postural control through the activation of ventro-medial and dorso-lateral systems, as well as the importance of the coactivation mechanism between the two lower limbs (Dietz et al., 2002) to keep the body CoM over the feet (Dietz, et al., 1992; Dietz et al., 1989), justify the study of mechanisms that occur in both sides of the body in relation to a unilateral movement like gait initiation as well as in relation to the gait cycle.

Basic structures involved in the control of locomotion and postural muscle tone are located in the midbrain (Takakusaki, et al., 2004). Some circumscribed regions have been identified as relevant in activating and controlling the intensity of spinal locomotor CPG operation, maintaining equilibrium during locomotion, adapting limb movement to external conditions and coordinating locomotion to other motor acts (Armstrong, 1986; Jordan, 1986; Orlovsky, 1991). Among the main supraspinal centres involved are the sensorimotor cortex and the supplementary motor area (Kapreli et al., 2006; Mackay-Lyons, 2002; Miyai et al., 2002) the cerebellum, the basal ganglia (Garcia-Rill, 1986; Mackay-Lyons, 2002), the midbrain locomotor region (Kandel, et al., 2000; Mileykovskiy et al., 2000) and the spinal cord (Dietz, et al., 1992). The sensorimotor cortex is involved in the preparation for and execution of movement (Nelson, 1996). The cerebellum receives copies of CPG output to motoneurons via ventral spinocerebellar tract and spinoreticularcerebellar pathways, as well as information about the activity of the peripheral motor apparatus via the dorsal spinocerebellar tract (Orlovsky, 1991). Based on these, influences motoneurons indirectly via vestibulospinal, rubrospinal, reticulospinal and corticospinal pathways (Orlovsky, 1991). The cerebellum main role may be the timing of muscle activation, "fine-tuning" the output by adapting each step (Lansner & Ekeberg, 1994). Nevertheless, both the cerebellum and the basal ganglia seem to play an important role in timing of sequential muscle activation, with the basal ganglia operating at the level of planning, initiation, execution, and termination of motor programs as well motor learning (Mackay-Lyons, 2002; Wichmann & DeLong, 1996). The midbrain locomotor region activates "muscle tone excitatory system" and "rhythm generating system" (Takakusaki, et al., 2004). Although not being relevant in gait, the motor cortex is involved in the modification of CPG activity in unstable surfaces or when gait needs a visual orientation . The degree of supraspinal and spinal influences in movement generation is determined by context (Mackay-Lyons, 2002). The main structures involved in postural control in both standing and walking are represented in Figure 1.

2.2 The role of afferent information

2.2.1 Upright standing

The ability to reweight sensory information depending on the context is important to maintain stability when a person moves from one context to another (Peterka, 2002). For instance, while vestibular information may not be a large contributor for the control of upright stance (Winter et al. 1998) and for triggering or coordinating muscle activation patterns associated to ankle strategy (Horak, et al., 1990), it is likely to play a crucial role during moments of increased postural instability (Fitzpatrick and McCloskey 1994). As in normal conditions proprioceptive information assumes more relevance than other sources, in this paper focus has been given to the role of proprioceptive information (Figure 2).

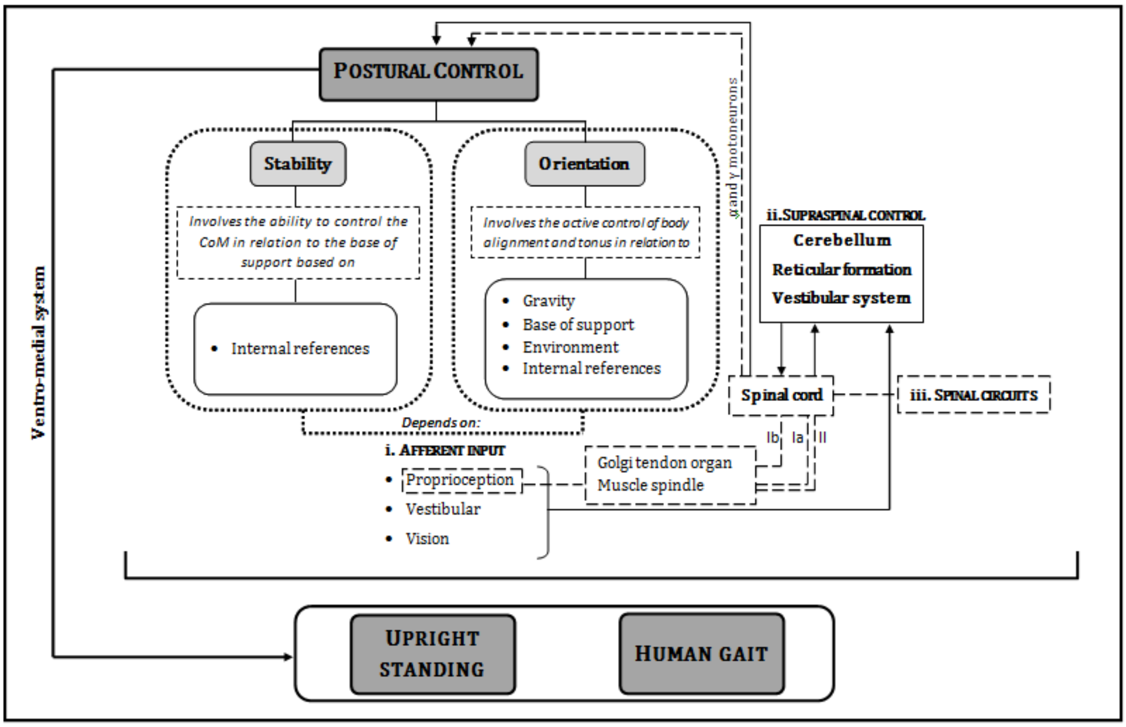

Figure 1: A conceptual schematic diagram illustrating the main structures involved postural control in both standing and walking.

It is well known that the mechanoreceptors (i.e. specialised sensorial receptors responsible for transduction of mechanic events into neural signs (Grigg, 1994)) accounting for proprioceptive information are primarily founded on muscles, tendons, ligaments and capsule (Hogersvorst & Brand, 1998; Jami, 1992; Johansson et al., 1991). Receptors located in the deeper skin tissue and fascia are traditionally associated with touch receptors, being categorised as additional sources (Edin & Johansson, 1995; Grigg, 1994; Macefield et al., 1990). Support has been given to the role of the Golgi tendon organs in providing afferent input from "gravity-dependent" receptors required to indicate the projection of the body's CoM within the base of support (Dietz, 1996; Dietz, 1998; Dietz & Colombo, 1996; Dietz, et al., 1992). Also, the small magnitudes of sway observed during quiet standing may be enough to alter muscle lengths, resulting in changes of Ia-afferent input onto the motoneuron pool of the lower limbs. Recent studies by (Loram et al., 2005b) have suggested this possibility, whereby muscle length changes in the gastrocnemius and soleus muscles during quiet standing have been detected within the range at which muscle spindles are sensitive to movement (Proske et al., 2000). Support has been given to the role of medium latency responses from group II during standing (Corna, et al., 1996; Nardone, et al., 1996; Schieppati, et al., 1995). Indeed, there is evidence that muscle spindle type II fibres play a more relevant role than group Ia fibres in the control of bipedal stance (Marchand-Pauvert, et al., 2005) as only medium latency responses have a stabilising effect during perturbations of stance, and also because these fibres are more influenced by the "postural set" (Nardone, et al., 1990). Findings obtained by Nardone et al, 1996, demonstrate the existence of crossed neural pathways fed by these fibres, which explains the bilateral electromyographic responses to unilateral perturbations during standing. This finding is supported by (Dietz, 1996), as this author argues that a complex bilateral coordination of leg muscle activation (mediated by a spinal mechanism (Dietz & Berger, 1984)) is needed for upright postural control during locomotion.

All the receptors mentioned above, and the corresponding afferents input, may allow a modulation of postural activity in relation to muscle length and tension variation, but only a combination of afferent inputs can provide the necessary information to control body equilibrium (Dietz, 1996). The role of proprioceptive information from ankle muscles has been highlighted in various studies (Fitzpatrick, et al., 1994; Fitzpatrick, Gorman, et al., 1992; Gatev, et al., 1999; Loram, et al., 2005a). Some authors go further, arguing that normal subjects can stand in a stable manner when receptors of the ankle muscles are the only source of information about postural sway (Fitzpatrick, et al., 1994; Fitzpatrick, Gorman, et al., 1992). The soleus and the gastrocnemius have traditionally been considered the source of muscle proprioceptive information signalling changes in body position (Fitzpatrick, Taylor, et al., 1992; Loram & Lakie, 2002a; Loram, et al., 2005b). These muscles act predominantly as active agonists and, because the foot is constrained on the ground, they prevent forward toppling of the body, whose centre of gravity is maintained in front of the ankle joint (Fitzpatrick, Gorman, et al., 1992; Lakie, et al., 2003; Loram & Lakie, 2002a; Loram, et al., 2005a; Maki & Ostrovski, 1993). The problem with muscle spindles as position sensors is that they are able to generate impulses in response to muscle length changes as well as from fusimotor activity (Proske, 2006). According to (Di Giulio, et al., 2009) the best proprioceptive information may come from un-modulated muscles crossing the joint in parallel with the active agonist. In fact, earlier studies stated that, depending upon the stance conditions, muscle stretch does not necessarily result in a compensatory stretch reflex response but instead results in an antagonistic muscle activation (Gollhofer et al., 1989; Hansen et al., 1988). Based on this, it has been argued that reciprocal patterns of muscle activation are typically involved in postural control (Di Giulio, et al., 2009; Latash, 1993). Neurophysiologically, reciprocal inhibition is mediated, at least in part, by a dysinaptic circuit in the spinal cord that is subject to several supraspinal as well as segmental modulator mechanisms (Jankowska, 1992) and varies according to the way in which antagonist muscles are activated (Lavoie, et al., 1997). Synergies between antagonist muscles include simple patterns of reciprocal activation, co-contractions, and complex triphasic activation patterns (Lavoie, et al., 1997). There is evidence that the strength of dysinaptic inhibition is reduced during co-contraction of antagonist muscles compared with reciprocal activation (Nielsen & Kagamihara, 1992). Another source of proprioceptive information may come from the cutaneous afferents of the feet as there is a large distribution of cutaneous receptors at various locations on the sole of the foot (Kennedy & Inglis, 2002). It has been suggested that this source of proprioceptive information contributes to both the coding and spatial representation of body posture during standing (Roll, et al., 2002) and that the architecture and physiology of the foot appear to contribute to the task of bipedal postural control with great sensitivity (Wright, et al., 2012)

2.2.2 Human gait

During gait, afferent feedback adapts dynamically, through a reciprocal relationship, the response of the CPG to environmental requirements and assumes multiple roles in regulating the production of motor patterns, such as: (1) the production of detail in the temporal pattern of muscle activation sequence (Ivanenko et al., 2006; Pearson, 1993), (2) the reinforce of ongoing motor activity, particularly those involving load-bearing muscles, such as the extensor muscles during the stance phase of gait (Pearson, 1993; Sinkjær, et al., 2000; Stephens & Yang, 1996), and (3) the control of transition from one phase of movement to another (Lacquaniti et al., 1999; Pearson, 1993). Swing is initiated when the leg is extended (stretching the flexor muscles) and unloaded (reduced force in extensor muscles sensed by the Golgi tendon organ of the extensor muscles) (Zehr & Duysens, 2004). Consequently, gait cycles depend on the afferent input from peripheral receptors as the muscle force production at a given level of motor unit recruitment can change according to length (velocity) and tension variations (Frigo et al., 1996).

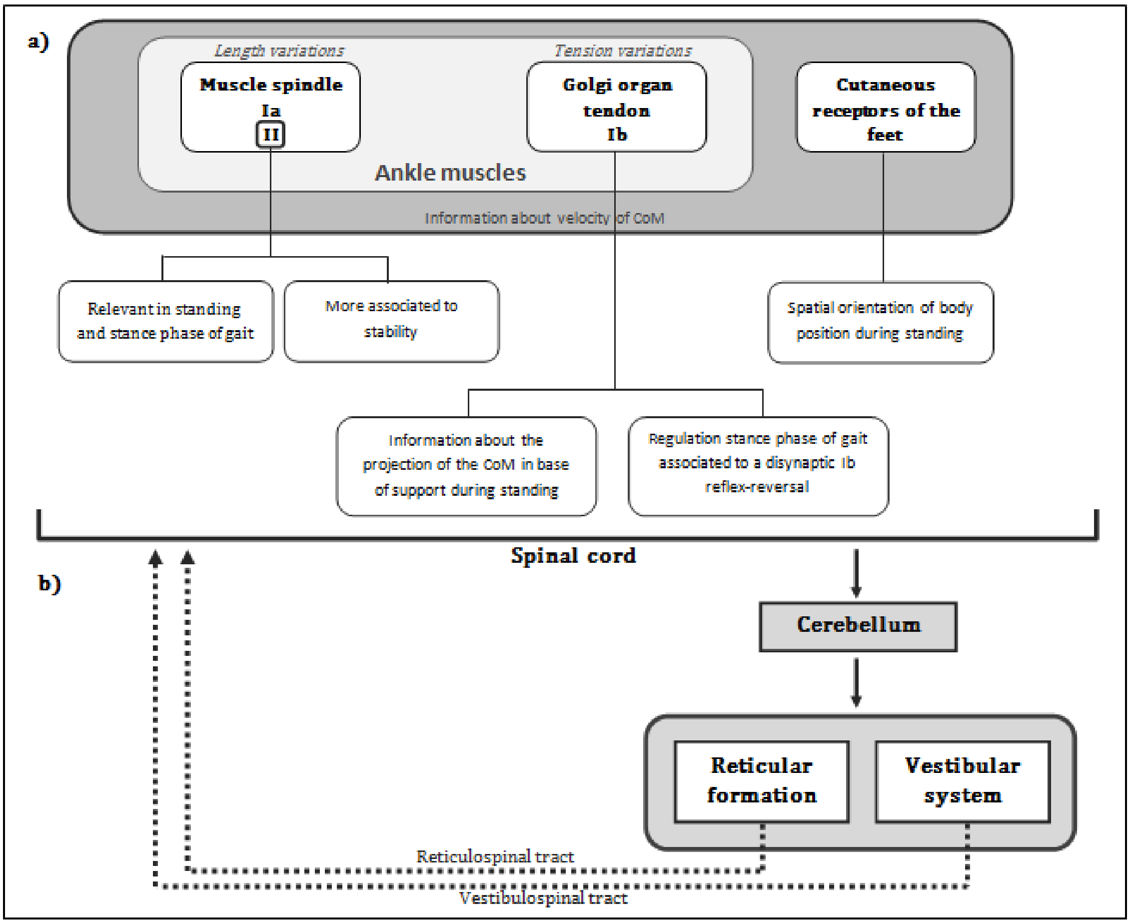

Figure 2: Representation of the most important proprioceptive receptors and afferences and their role in standing and stance phase of gait (a). There are also represented important networks related to proprioceptive information (b). In this part of illustration (b), dotted lines represent efferent pathways and solid lines represent afferent pathways.

The monosynaptic excitation of spinal motoneurons from the large diameter group Ia afferent fibres related to a short latency response (Matthews, 1991) has been demonstrated when an expected stretch of the ankle extensors is imposed during gait (Sinkjaer et al., 1996; Yang et al., 1991). In addition, a phasic modulation of Ia input has also been demonstrated by changes in the magnitude of H-reflex over the course of the gait cycle, with the greatest attenuation occurring during flexion (Schneider et al., 2000; Yang & Whelan, 1993). This modulation is consistent with the fact that the maximum soleus length occurs during the foot off, when maximum plantar flexion of the foot occurs, which is coincident with its maximum force production (Orendurff et al., 2005). The modulation of the H-reflex is a reflection of: (1) the background excitability of the motoneuron pool, (2) the modulation associated with the activation of the antagonist muscle, and (3) presynaptic inhibition of the primary afferents (Yang & Whelan, 1993) that seems to be related partially to Ia afferents from the hip and knee extensor muscles (Brooke et al., 1997). Medium latency response from group II has been demonstrated during gait (Dietz et al., 1985) and some authors argue that this group is more important to feedback in the stance phase than group Ia (Grey, et al., 2001; Grey, et al., 2002; Nielsen & Sinkjaer, 2002; Sinkjær, et al., 2000). Earlier studies have suggested that strong central effects of group II muscle afferents are mediated via a complex neural pathway influenced by supraspinal input and peripheral input during walking (Dietz et al., 1987; Yang, et al., 1991). Specifically, there is evidence for the role of vestibulo- and reticulo-spinal pathways (Davies & Edgley, 1994) that supports the hypothesize that the facilitation of the relevant lumbar propriospinal neurons by descending tracts neurons would be stronger over group II during maintenance of posture than during voluntary contractions (Marchand-Pauvert, et al., 2005). Also, the role of group Ib load-sensitive afferences related to a medium latency response has been reported to contribute to the regulation stance phase of gait (Stephens & Yang, 1999) associated to a disynaptic Ib reflex-reversal (Stephens & Yang, 1996). Findings reported in (Grey et al., 2007) suggest that tendon organ feedback via an excitatory group Ib pathway contributes to the late stance phase enhancement of the soleus muscle activity. The combination of the different afferent inputs plays an important role on gait dynamics related to the ipsilateral limb but also on the contra-lateral limb, as it has been demonstrated that unilateral leg displacement during gait evokes a bilateral response pattern, with a similar onset on both sides (Dietz & Berger, 1984). From a functional point of view, this interlimb coordination is necessary to keep the body's CoM over the feet (Dietz, 1996).

2.3 Biomechanical aspects

2.3.1 Upright standing

Upright stance is associated with small deviations from an upright body position, which results in a gravity-induced torque acting on the body, causing it to accelerate further away from the upright position. Corrective torque must be generated to counter the destabilizing torque due to gravity. This process of continuous small body deviations countered by corrective torques creates a pattern known as spontaneous sway. The mechanisms underlying spontaneous sway are not fully understood, and controversy remains regarding the organisation of sensory and motor systems contributing to the spontaneous sway. Numerous authors have suggested that active feedback control mechanisms contribute to the maintenance of upright stance (Fitzpatrick et al., 1996; Johanson & Magnusson, 1991; Peterka & Benolken, 1995; Peterka & Loughlin, 2004; van der Kooij et al., 2001). Recent studies have shown that a model based primarily on a feedback mechanism with a 150 to 200 ms of delay can account for postural control during a broad variety of perturbations (Peterka, 2002; Peterka & Benolken, 1995; Peterka & Loughlin, 2004) and can yield a spontaneous sway pattern that resembles normal (Peterka, 2000) or pathological spontaneous sway (Parkinson’s disease; (Maurer, et al., 2003)). However, the relevance of feedback mechanisms for postural control is still debated. Some authors concluded from their experiments that corrective torque originating from feedback control is insufficient for stabilizing the body (Fitzpatrick, et al., 1996). Others suggested additional sources for corrective torque, like prediction (Morasso, et al., 1999; van der Kooij, et al., 2001), or have proposed more complex concepts (Baratto, et al., 2002; Collins & De Luca, 1993; Loram & Lakie, 2002b). Postural sway has been viewed as a result of a correlated random-walk process (Collins & De Luca, 1993), a result of computational noise (Kiemel et al., 2002), and/or a moving reference point (Zatsiorsky & Duarte, 1999). The possible importance of the postural sway as a reflection of a hypothetical search process within the system of postural stabilization has been emphasised (Mochizuki, et al., 2006; Riley, et al., 1997).

From a functional point of view, the control of human upright posture stability is commonly viewed as a continuous stabilization process of a multilink inverted pendulum, where the main controlled parameter is the CoM position within the limits of the supporting base (Maurer & Peterka, 2005). This aspect has been described as biomechanical constraints that determines patterns of postural coordination (Buchanan & Horak, 2003). In stance, the limits of stability, i.e., the area over which individuals can move their CoM and maintain equilibrium without changing the base of support, are shaped like a cone (McCollum & Leen, 1989). Thus, equilibrium is not a particular position but a space determined by the size of the support base and the limitations on joint range, muscle strength and sensory information available to detect limits. The CNS has an internal representation of this cone of stability that it uses to determine how to move to maintain equilibrium (Horak, 2006). Gatev et al., 1999, reported a significant correlation between spontaneous body sway and the activity of the gastrocnemius muscle. They also found that gastrocnemius activity preceded temporally CoM displacement, suggesting a central program of control of the ankle joint stiffness working to predict the loading pattern. More recent studies proposed that the actual postural control system during quiet standing adopts a control strategy that relies notably on velocity information of CoM and that such a controller can modulate muscle activity in an anticipatory manner without using feedforward mechanisms (Masani et al., 2003). According to this view, velocity feedback can play a significant role in anticipating body position changes because it carries information about the subsequent state of the body, i.e., a change in CoM velocity indicates the direction and intensity with which the current CoM displacement will be changed in the following time instant. It has been hypothesised that the integration of proprioceptive and plantar cutaneous sensations would play a significant role in the velocity feedback mechanism (Masani, et al., 2003). Another biomechanical constraint is related to frequency of postural sway (Nashner et al., 1989), as when postural sway is lower than 0.5 Hz the body can be compared to a simple inverted pendulum (McCollum & Leen, 1989), and when it is higher than this value, the body can be compared to a double inverted pendulum with the fulcrum at the hip level (Yang, et al., 1990).

2.3.2 Human gait

The coordination between posture and movement involves the dynamic control of the CoM in the base of support (Stapley, et al., 1999). Consequently, to access the simplified concept of locomotion it is necessary to consider the behavior of the CoM during gait cycles. The trajectory described by the CoM in the plan of progression is a sinusoidal curve that moves vertically twice during one cycle and laterally in the horizontal plan, and that is similar in form to that found in the vertical displacement (Gard et al., 2004; Norkin & Levangie, 1992). Peak-to-peak amplitude is described as being about 4-5 cm for adults at freely chosen speed and has been used to estimate exchanges of mechanical energy, efficiency, work, and to describe the symmetry as an indicator of the quality of gait (for more information see (Gard, et al., 2004)).

The human gait results from a complex interaction of muscle forces, joint movements and neural commands. Variables, including electromyographic activity, muscle torque, ground reaction forces, kinematics and metabolic-energy costs have been assessed and quantified. This data set requires an interpretation and organisation of the fundamental principles that elucidate the mechanisms of gait. Several models have been suggested to describe human gait mechanisms (Cavagna, et al., 1977; Cavagna & Margaria, 1966; Donelan, et al., 2002b; Kuo, et al., 2005, 2007; Saunders et al., 1953; Waters & Mulroy, 1999). The six determinants of gait theory (Saunders, et al., 1953), based on the premise that vertical and horizontal CoM displacements are energetically costly, proposes a set of kinematic features that help to reduce CoM displacement. However, there is evidence that some determinants have a non-significant role on the CoM vertical displacement and that there is higher metabolic expenditure when subjects voluntary reduce vertical displacement of CoM (for review see (Kuo, et al., 2007)). The inverted pendulum model proposes that most of the work during gait is performed by a passive mechanism of exchange of gravitational potential and kinetic energies (60-70%) (Cavagna, et al., 1977; Griffin, et al., 2003). However, this model cannot reproduce the existence of two peaks in vertical ground reaction force (Pandy, 2003; Zajac, et al., 2003) and does not account for the costs which are not considered responsible for work, like isometric force for stabilisation and body weight support (Kuo, et al., 2005). The difference in the percentage of energy recovery in relation to an ideal inverted pendulum has been related mostly to double support phase (McGeer, 1990). Indeed, it has been demonstrated a low percentage of energy recovery in the double support phase (Geyer et al., 2006) related to the interruption of the energy-conserving motion of single support by an inelastic collision of the swing leg with the ground, leading to changes in velocities of the legs and the CoM (Kuo, et al., 2007). This energy loss can be reduced by 75% through the application of a propulsion impulse in the trailing leg immediately before collision of the leading leg (Kuo, 2002). Simulations suggest that the ankle plantar flexor (soleus, gastrocnemius) and the uni- and bi-articular hip extensors (gluteus maximus, hamstrings) dominate work output over the gait cycle (Neptune, Kautz, et al., 2004). These muscles, being active in the late stance and in the beginning of stance, are therefore restoring energy to the body near double-support (Zajac, et al., 2003).

Ankle plantar flexors are the primary contributors for forward progression and vertical support (Kepple et al., 1997), before midstance, they hinder progression (Neptune, et al., 2001) and during midstance, they maintain body support and the forward motion of the trunk and leg, which is consistent with inverted-pendulum-like ballistic walking as the synergy of these muscles in this subphase occurs with minimal metabolic energy expenditure, as expected in ballistic-like walking (Zajac, et al., 2003). Biarticular hip extensors generate forward acceleration during the first half of stance, while uniarticular quadriceps muscles and the uniarticular hip extensors decelerate the body mass centre and provide body support (Liu, et al., 2006; Neptune, Kautz, et al., 2004). The biarticular quadriceps muscle is a significant contributor to forward progression in late stance (Neptune, Kautz, et al., 2004).

According to Donelan et al., 2004, lateral stabilisation exacts a modest metabolic cost as walking requires active lateral stabilisation. It has been demonstrated that the gluteus medius, although acting primarily outside the sagittal plane in walking, contributes to support and slows progression (less than the other muscles) in the first half of stance and contributes to support in the second half (Liu, et al., 2006). Additionally, it has been demonstrated that the body lateral motion is partially stabilised via medio-lateral foot placement (Donelan, et al., 2004; Kuo, 1999).

3. MOVEMENT EFFICIENCY

The relationship between muscle activity and whole body mechanics is too variable and complex to allow direct control of the later without an intermediate kinematic representation (Lacquaniti, et al., 1999). There is evidence that supports the idea that global kinematic gait is controlled (Ivanenko et al., 2004). Kinematics is relatively invariant in various modes of locomotion, while the electromyographic activity patterns to produce the required kinematic patterns can vary considerably (Grasso et al., 1998; Ivanenko, et al., 2004). These findings suggest that neural circuitry can somehow specify limbs kinematics, and the appropriate muscle synergies would be determined in a subordinate and flexible manner to adapt to the current mechanical constraints (Lacquaniti, et al., 1999; Lacquaniti et al., 2002). The basic biomechanical control signal may exert its action through an appropriate model of inverse dynamics and feedback device that determines the muscle torque necessary to achieve kinematic patterns (Ivanenko, et al., 2004). The significance of muscle redundancy would then be to allow the same movement to be carried out by means of different combinations of muscle activity under dissimilar environmental circumstances, for instance, to cope with fatigue or changes in load (Lacquaniti, et al., 1999).

The major function of muscles in gait is to generate and absorb energy; such function is largely ignored in neurophysiological research (Winter & Eng, 1995). The body has the capacity of transferring energy between segments across the joint centres and can store and recover energy in the passive elastic tissues in the tendon and muscles. However, this last energy conserving mechanism is quite small in walking (Winter & Eng, 1995). The CNS has learnt how to create motor patterns to conserve much of the energy that was generated earlier in the gait cycle. It has been estimated that of the total energy changes of all body segments over the gait cycle only 33% are caused by active muscle generation and absorption, while 67% are due to the passive transfers between segments (Pierrynowski et al., 1980). Considering this it is important to quantify the movement also on the criterion of efficiency (Fetters & Holt, 1990; Sparrow & Newell, 1998).

In biomechanical and physiological research, efficiency of movement is normally defined as the ratio of the mechanical work performed and the metabolic cost of performing the work (Stainsby et al., 1980). Typically, the efficiency is calculated as:

$$Efficiency\ (\%) = \frac{Mechanical\ work}{Energy\ work} \times 100$$

Energy expenditure during walking can be characterised through mechanical energy estimations (Cavagna et al., 1963; Saibene & Minetti, 2003; Willems, et al., 1995) or metabolic energy measurements (Waters & Mulroy, 1999). Mechanical energy is generally estimated by one of three approaches: (1) analysis of energy changes of the CoM in relation to the surroundings (external work) and of the body segments regarding the CoM (internal work) (Cavagna & Margaria, 1966; Cavagna, et al., 1963; Willems, et al., 1995); (2) analysis of the energy changes of moving body segments (sum of segmental energies) or (3) measurement of muscle power around the joints (net joint work) (Winter, 2005). In all mechanical energy estimations, the actual amount of work performed is underestimated as additional metabolic work resulting from isometric muscle contractions or antagonist co-contractions is not taken into account (Fetters & Holt, 1990; Winter, 2005). This problem is overcome when assessing metabolic energy, i.e., by measuring oxygen consumption during walking (Fetters & Holt, 1990; Vandewalle, 2004). The relation between metabolic cost and the mechanical work performed by stance limb muscles to lift and accelerate the CoM during walking has been already demonstrated (Donelan et al., 2001; Donelan, et al., 2002a) and has been considered a valid predictor of walking performance (Anderson & Pandy, 2001). Metabolic energy expenditure can be accessed through indirect calorimetry, where oxygen consumption and/or carbon dioxide production is measured and converted into energy expenditure using formulae (Cunningham, 1990; Garby & Astrup, 1987) which have been reported as a valid method (Levine, 2005). Mechanical and metabolic energy analyses allow monitoring how the CNS takes advantage of energy conserving mechanisms in order to achieve a more efficient movement.

4. CONCLUDING REMARKS

Postural control has been vastly explored in the scientific community. However, the complexity of the interrelations between neural and mechanical aspects and environment leads to the need of studying postural control in a holistic way. In addition, the study of postural control needs to reflect the dynamic inter-relation of the different components of human movement on the basis of movement efficiency. The adaptability, vulnerability and continuous dependency of afferent information on the postural control system turn this area a focus of clinical interest. Considering that the postural control system has the capacity of reorganisation for higher movement performance, it is important to understand in detail the static and dynamic postural control mechanisms and strategies and how these mechanisms influence other systems and are influenced by changes in afferent and efferent information.

PART B – *BOOK* CHAPTER

Surface electromyographic amplitude normalization methods: a review

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ABSTRACT

The electromyogram is the summation of the motor unit action potentials occurring during contraction measured at a given electrode location. The voltage potential of the surface electromyographic signal detected by electrodes strongly depends on several factors, varying between individuals and also over time within an individual. Thus, the amplitude of the EMG signal itself is not useful in group comparisons, or to follow events over a long period of time. The fact that the recorded electromyographic amplitude is never absolute is mainly because impedance varies between the active muscle fibers and electrodes and its value is unknown. The EMG signal is highly variable and is dependent upon many factors. Thus, the amplitude of the temporally processed electromyography can only be used to assess short-term changes in the activity of a single muscle from the same individual when the electrode setup has not been altered. To allow comparison of activity between different muscles, across time, and between individuals, the EMG signal should be normalized, i.e. expressed in relation to a reference value obtained during standardized and reproducible conditions.

Notwithstanding the importance of electromyographic amplitude normalization, studies on functional activities, such as gait, do not seem to show a uniform methodology. Taking this into account, the main purpose of this chapter is to review and discuss different normalization procedures to relate the most appropriate method for specific situations, based on how the normalization method might influence data interpretation. In addition, this review supports the development of proper normalization procedures for biomechanical studies of functional activities like human gait.

Keywords: biomechanics, electromyography, isometric actions, dynamic actions, isokinetic actions, human gait

1. INTRODUCTION

Electromyography (EMG) is unique in specifying muscle activation. Specifically, surface EMG is a convenient index of muscle excitation and allows a description of muscular patterns (Bouisset & Do, 2008). Analysis of amplitude modulation is usually performed with the signal envelope (rectification and low-pass filtering) or by estimation of the average rectified or root-mean-square value with a sliding window (Campanini et al., 2007). However, absolute EMG amplitude values are not reliable, due to many factors which can influence them (Farina et al., 2004). Variance of the estimate can be substantially reduced with special techniques, such as signal whitening and multichannel processing (Campanini, et al., 2007). Nevertheless, the main limitation in the interpretation of EMG amplitude results not from processing algorithms but from the masking effects of unwanted factors.

The use of amplitude modulation for the assessment of relative muscle activation during movement relies on two main requirements: 1) EMG amplitude should be directly related to the level of excitation sent to the muscle from the spinal cord (Bonato, 2001), and 2) amplitude should not be influenced by factors other than the excitation level (Bonato et al., 2001). Both requirements are difficult to satisfy during dynamic contractions: Amplitude is not directly related to the excitation level because of amplitude cancellation (Farina, et al., 2004). Moreover, the relation between amplitude and excitation level depends on the pattern of motor unit activation (Fuglevand et al., 1993), electrode location in relation to innervations zones and tendon regions, and crosstalk. In dynamic contractions, volume conductor properties (Mesin et al., 2006) and the relative position of the electrodes with respect to muscle fibers may vary over time; therefore, amplitude may be additionally influenced by geometrical factors, in a subject- and muscle-specific way. Quantitative comparisons of patterns of EMG amplitude during movement across muscles or subjects should consequently require analysis of the possible confounding factors.

2. IMPORTANCE OF NORMALIZATION PROCEDURES

The electromyogram is the summation of the motor unit action potentials occurring during the contraction measured at a given electrode location. This activity is often expressed in millivolts, but other units can be output by the acquisition device. EMG normalization is the process by which the electrical signal values of activity are expressed as a percentage of that muscle's activity during a calibrated test contraction (Lehman & McGill, 1999). Aiming to improve absolute EMG reliability and to provide an expression of relative muscle activation, the normalization of EMG data requires the use of a standardized and reliable reference value against which experimental data are measured (Burden, et al., 2003).

The amplitude and frequency characteristics of the raw EMG acquired using surface electrodes has been shown to be sensitive to many intrinsic and extrinsic factors (De Luca, 1997). As such, the amplitude of the temporally processed EMG can only be used to assess short-term changes in the activity of a single muscle from the same individual when the electrode setup has not been altered (Mathiassen, 1997; Mathiassen et al., 1995). To allow the comparison of activity between different muscles, across time, and between individuals, the EMG should be normalized (De Luca, 1997; Knutson, et al., 1994; Mathiassen, et al., 1995; Mirka, 1991; Yang & Winter, 1984), i.e. expressed in relation to a reference value obtained during standardized and reproducible conditions (Mathiassen, et al., 1995).

The fact that the acquired EMG amplitude is never absolute is mainly because the impedance varies between the active muscle fibers and electrodes and its value is unknown (Gerdle et al., 1999). The EMG is highly variable and is dependent upon electrode application and placement (Jensen et al., 1993), perspiration and temperature (Winkel & Jørgensen, 1991), muscle fatigue (Hansson et al., 1992), contraction velocity and muscle length, cross talk from nearby muscles (McGill & Norman, 1986), activity in other synergists and antagonists (Mathiassen & Winkel, 1990), subcutaneous fat thickness, and slight variation in task execution (McGill, 1991), to name a few. It would be impossible to control all these modulators of EMG amplitude in a clinical setting. Therefore, when comparing amplitude variables between measurements, normalization of some kind is required, i.e. the EMG signal is converted into a scale that is common to all measurement occurrences. Normalization controls for the aforementioned variables and facilitates the comparison of EMG signals across muscles, between subjects, or between days for the same subject. By expressing the neural activity (EMG amplitude) as a percentage of the reference task, interpretation of the signal is moved into a framework of biological significance (Lehman & McGill, 1999).

3. AMPLITUDE NORMALIZATION METHODS

As already mentioned, because of the inherent variability of EMG signal, clinical interpretation of surface EMG requires the normalization of the EMG signal for physiological interpretation and for comparison between muscles and between subjects. Previous studies have used a number of different methods to produce reference EMG values for normalization purposes that can be repeated across participants and test days, including isometric, isokinetic and dynamic muscle actions (Burden, et al., 2003; Lehman & McGill, 1999; Yang & Winter, 1984), Figure 1.

3.1 Isometric actions

3.1.1 Maximal and submaximal voluntary contraction methods

Typically, EMG is expressed as a percentage of the maximum neural drive acquired while a subject performs an isometric maximal voluntary contraction (MVC) of the desired muscle. This is perhaps the most powerful strategy for physiologic interpretation in healthy people. However, maximal effort contractions are not usually possible for older patients or for patients with symptoms. Also, acquiring maximal electrical activity is not always achieved during an effort involving maximal force generation (Lehman & McGill, 1999).

The use of MVC has several technical concerns, which have an impact on the validity and reliability of the normalization protocol, associated with isometric testing. These concerns include the inertial effects at the onset of the test, and the patient fatigue, posture and motivation. Furthermore, normalization is not a measure of muscular tension, but is a measure of muscular activation expressed as a percentage of activity relative to the subject's MVC (Soderberg, 1992). Therefore, the EMG from an isometric MVC may not represent the maximum activation capacity of the muscle either at lengths other than those at which the MVC was performed, or under non-isometric conditions, as was shown by (Mirka, 1991). Additionally, the other major limitation of using the EMG from an MVC as the denominator in the normalization equation concerns the poor reliability of EMG signal that has been reported from such contractions (Clarys, 2000; Perry, 1992; Yang & Winter, 1983).

In spite of the aforementioned limitations, maximal isometric muscle actions are the suggested method of normalizing by SENIAM and Kinesiology's guidelines and are the most widely employed normalization method (Burden, et al., 2003; De Luca, 1997). Despite some studies demonstrating good EMG reliability between days (Hsu et al., 2002) and acceptable EMG reliability either between days or between weeks (Ball & Scurr, 2010), the majority of research has shown poor EMG reliability both within and between subjects and between sessions for isometric EMG levels of different muscles, particularly at maximal loads due to fatigue onset (Ball & Scurr, 2010; Bamman et al., 1997; Heinonen et al., 1994; Yang & Winter, 1983), synergistic contribution and psychological factors (Enoka & Fuglevand, 1993; Miaki et al., 1999; Yang & Winter, 1983). Due to this instability of the EMG signal at near maximal levels, in (De Luca, 1997) it is recommended that EMG amplitudes are normalized to force levels that are 80% of the maximum voluntary muscle action. Previous research has demonstrated that sub-maximal loads produced improved reliability between days compared to maximal loads for knee extensors and triceps (Rainoldi et al., 1999; Yang & Winter, 1983).

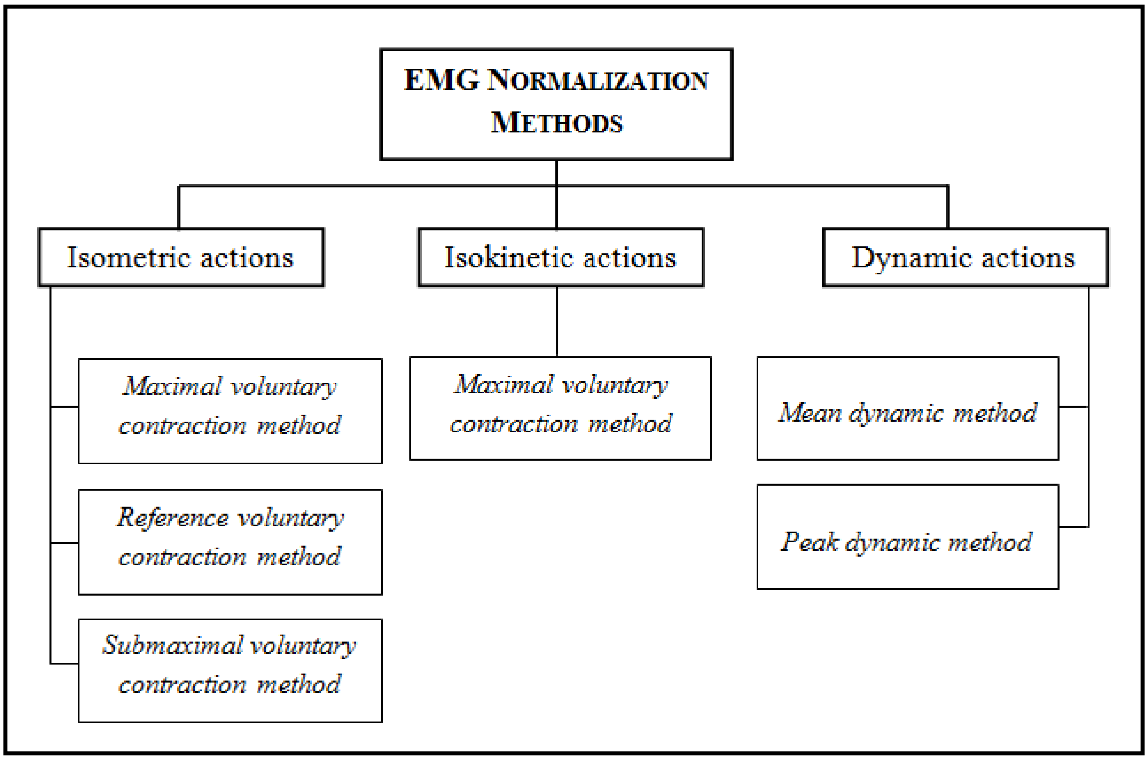

Figure 1: Usual electromyographic normalization methods.

All the aforementioned methods provide an output that relates the task EMG to the EMG obtained during a particular standardized event and, as such, were termed as bioelectric normalizations in (Mathiassen, et al., 1995). An alternative manner of normalization involves translating the EMG that forms the denominator of the equation in the isometric methods into a force or torque variable. Typically, the EMG is related to a maximal contraction, or a submaximal contraction at a known level of force. One purpose of such biomechanical normalization methods is to generate an estimate of the physical load on the muscle under investigation (Marras & Davis, 2001; Mathiassen, et al., 1995).

3.1.2 Reference voluntary contraction method

Controlled reference voluntary contractions (RVC) postures are interesting for clinical populations who are unable to attempt maximal efforts or who need an analogous controlled task for interpreting repeated tests. For example, standing upright holding 5 kg in the hands with the arms outstretched horizontally during each test session will produce a very similar low back moment day after day. Any change in un-normalized EMG amplitude could be due to any of the modulators and artifacts noted in the previous section. Any change in EMG amplitude (normalized to this RVC) indicates a true increase or decrease in the neural drive (Lehman & McGill, 1999).

3.2 Dynamic muscle actions

Gait EMG signal was first normalized using a method that divided each point that constitutes the processed EMG by the peak value acquired from the same EMG. This method, subsequently referred to as the peak dynamic method, still appears to be popular among gait electromyographers (e.g. (Arendt-Nielsen et al., 1996; van Hedel et al., 2006)). In (Yang & Winter, 1984) a number of normalization methods are compared in an attempt to find the one which could provide a normal gait EMG template and, therefore, improve the use of electromyography as a diagnostic tool in gait analysis. Based on this rationale, the criterion for selecting the best method was the one that most reduced the inter-individual variability of the ensemble averaged EMG signal. The authors concluded that the mean and peak dynamic methods would help reduce the subject-specific and situation-specific conditions that may increase signal variance. They also pointed out that using these methods came at the expense of information inherent on the variance of the EMG signal. In (Ball & Scurr, 2010) it was found that squat jump and sprint provided reliable EMG amplitudes both between days and between weeks for all muscles of the triceps surae. However, dynamic tasks such as reaction tests (Horstmann et al., 1988), sub-maximal running, one-leg hopping, drop jumps (Gollhofer et al., 1990) have shown poor EMG reliability between testing sessions.

3.3 Isokinetic actions

Electromyography amplitudes from an isokinetic muscle action have been proposed as an alternative to isometric muscle actions for EMG normalization to allow joint angle, torques and corresponding EMG amplitudes to be quantified (Kellis & Baltzopoulos, 1996; Mirka, 1991). Good EMG reliability has been shown between trials for isokinetic exercises for the knee extensors and flexors (Finucane et al., 1998; Larsson et al., 2003) and inappropriate reliability has been shown between isokinetic exercises for the triceps surae muscles (Ball & Scurr, 2010).

4. EMG AMPLITUDE NORMALIZATION DURING GAIT

Early investigations of dynamic tasks, including walking (Arsenault, Winter, et al., 1986a; Dubo et al., 1976), have used the EMG from an isometric MVC as the normalization reference value. However, it is generally recognized that the EMG from an isometric MVC is less reliable than the signal obtained from an isometric submaximal contraction (Yang & Winter, 1983), and that it might not represent the maximum activation capacity of the muscle (Enoka & Fuglevand, 1993). This has led to the evaluation and use of other reference values; in addition, some authors have expressed alternative aims for EMG normalization (Winter & Yack, 1987a; Yang & Winter, 1983). As already mentioned, (Yang & Winter, 1984) compared four different normalization reference values to identify which one would result in the greatest reduction in inter-subject variability during walking. The use of either the mean or the peak linear envelope from the ensemble average reduced the inter-subject coefficient of variation in relation to the un-normalized data in all five lower limb muscles analyzed. In comparison, the inter-subject coefficient of variation was generally increased by using either 50% of the isometric MVC or the mean EMG per unit of isometric moment as the reference values. A reduced inter-subject coefficient of variation was also demonstrated for the biceps brachii during isotonic elbow flexions and extensions (Allison, et al., 1993) and for the gastrocnemius during a balancing task (Knutson, et al., 1994) by using the peak or mean ensemble value in comparison to the EMG from an isometric submaximal contraction (Allison, et al., 1993) or MVC (Allison, et al., 1993; Knutson, et al., 1994).

Although the peak and mean ensemble methods are the only feasible ways of normalizing EMG signal from patients with neurologic disorders (Yang & Winter, 1984), such methods tend to produce a normal EMG template for a particular task and, therefore, may remove the true biological variation within a group (Allison, et al., 1993; Knutson, et al., 1994). While the isometric MVC method is the only one that aims to reveal the percentage of the maximum activation capacity of the muscle required to perform a specific task (Yang & Winter, 1984), generally, the other methods mentioned above lead to changes in the un-normalized data as a consequence of variations in load and velocity of movement (Allison, et al., 1993).

In (Knutson, et al., 1994) the EMG activity of the gastrocnemius muscle was evaluated during gait using the MVC, mean dynamic method and peak dynamic method normalization in anterior cruciate ligament injured subjects. Data were compared statistically for the inter-class coefficient of variance (CV), variance ratio (VR) and intra-class coefficient of variance (ICC), being concluded that normalizing to MVC provided the most reproducible results based on the VR and ICC. However, this work did not identify how the normalization method might influence data interpretation and the justification for using the MVC method seems contradictory to that of (Yang & Winter, 1984) for rectus femoris, vastus lateralis, biceps femoris, tibialis anterior, and soleus muscles.

4.1 Mean dynamic method of normalization of the EMG signal during a full gait cycle

The mean dynamic method represents an average of both quiet and active periods during the gait cycle. Therefore, it may be more susceptible to systems with a low signal noise ratio, or it may represent baseline noise in movements that cause very phasic activation. This method, depicted in Figure 2, is more conservative as the overall variability in the signal may be reduced at the expense of true changes in activation level (Benoit, et al., 2003).

4.2 Peak dynamic method of normalization of the EMG signal during a full gait cycle

When representing the percentage of the peak dynamic method of EMG signal during repeated gait cycles (Figure 2), the procedure indicates the periods during the gait cycle at which the muscle is most active. However, it does not indicate the muscle's ability to activate. Therefore, the amount of activation cannot be related to any physiological measure and the patients' inability to contract the muscle due to pain inhibition, and altered neuromuscular performance, may not be observed (Benoit, et al., 2003).

4.3 Isometric maximal voluntary contraction method

Evidence suggests that the isometric maximal voluntary contraction (MVC) method best represents isotonic contractions at various speeds (Burden & Bartlett, 1999) and, with modeling, can estimate muscle forces during gait. However, adding to the disadvantages already mentioned, normalizing to isometric contraction does not represent dynamic contraction. The advantage of this technique is that normalization is based on the patients' ability to contract the muscle. Yet, this ability to perform a MVC is greatly affected by pain, and when the pain is inhibited with local analgesia postoperatively, there is an increase in the patients' ability to contract voluntarily the quadriceps to a maximal level (Arvidsson et al., 1986). The influence of pain-induced muscle inhibition would probably only affect the data when normalized to the MVC method. On the other hand, if the subject was unable to contract the muscle maximally during the MVC protocol, the relative amount of activation required during a cyclical movement such as gait might be represented by a change in the amount of activation recorded, and not merely by changes in temporal parameters. Although additional methods exist, such as using interpolated-twitch techniques (Rudolph et al., 2001) and using torque measurements to model the force output of the various muscle groups, these may not provide useful clinical information for rehabilitation purposes.

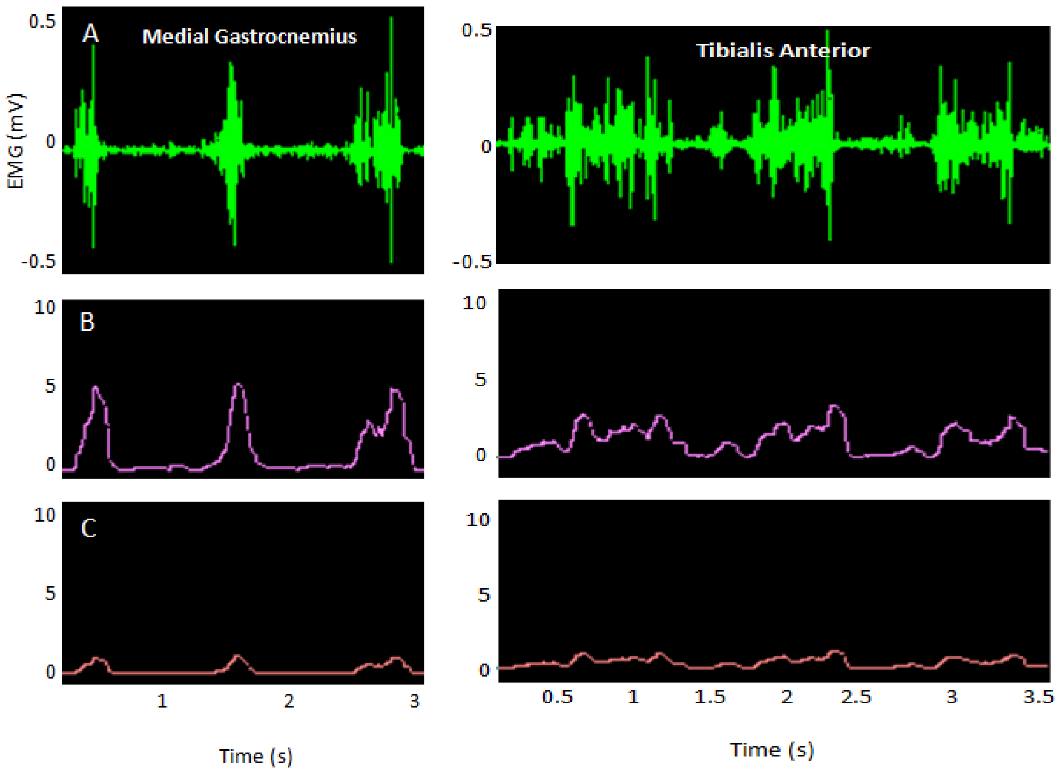

Figure 2: Raw EMG signal of medial gastrocnemius and tibialis anterior muscles obtained during gait at self-selected speed (A). The raw EMG signal was filtered and processed according to the root mean square (RMS) procedure and then normalized according to *mean dynamic* (B) and *peak dynamic* (C) methods.

All normalization methods exposed present advantages and limitations, Table 1. Normalization by the isometric of MVC is unreliable (De Luca, 1997) since muscle contraction during gait is mostly isotonic (Winter & Scott, 1991). The isokinetic MVC method has been used as a method to simulate with a higher degree of comparability to muscle contractions during gait. However, when compared to the isometric MVC method, it did not always produce satisfactory results, especially if one considers that it is better to have lower CV and lower VR, which represents the intra- and inter-individual variability of the EMG profile (Burden, et al., 2003). Another object of normalizing the EMG signal is to establish an average EMG profile to be a reliable template. Therefore, the peak dynamic method and the mean dynamic method have also been used (Winter & Yack, 1987b; Yang & Winter, 1984), in addition to the two normalization methods referred above. Additionally, it has been reported that profiles obtained with these methods are close to one another, and that the mean method produces better results with respect to reliability, as already mentioned. However, it has also been noted that normalization by using the peak and mean methods, which do not use reference values obtained from reference exercises, intentionally removes the true biological variation within a normal group (Allison, et al., 1993; Knutson, et al., 1994).

In (Nishijima et al., 2010), a different normalization method based on exercises under submaximal load (segment weight dynamic movement) is proposed. According to these authors, this method is as applicable as the isometric MVC method as a normalization method for establishing a gait EMG profile template. Moreover, for all of the eight muscles studied, the gait EMG peak amplitudes were lower than those obtained from the reference exercises of the segment weight dynamic movement method. Therefore, at least in terms of muscular activity level, being able to carry out the reference exercises may serve as a criterion of a person having a sufficient muscular activity level required for walking.

In Figure 3, the results of different EMG amplitude normalization methods are presented for rectus femoris (RF) activity during the propulsion phase of gait at self-selected speed. To access the EMG activity during isometric and isokinetic MVC the subject was positioned in closed kinetic chain on a quadriceps chair, under the following criteria: (i) hip and knee at 90º flexion; (ii) stabilization of the torso, the pelvis, right below the anterior superior iliac spine, and thighs; (iii) resistance applied 3 cm above the malleoli; (iv) arms crossed over the chest. Isometric MVC of RF muscle was performed by reaching maximal force as rapidly as possible and maintaining it for 3 seconds. Submaximal MVC was performed with 40% of isometric MVC and maintained for 3 seconds. Isokinetic MVC of the knee extensors was performed concentrically at 0.52 rad·s-1 interval up to 6.28 rad·s-1 between 90º and 0º of knee flexion. Reference contraction was obtained by asking the subject to hold a standardized load (3 kg). RF EMG activity during gait propulsion was expressed as a percentage of the peak RMS EMG from the isometric and isokinetic MVC, submaximal MVC and reference contraction. Dynamic repetitive movement exercises under the load of the segment weight (segment weight dynamic movement exercise (SWDM)) of the quadriceps femoris was performed with the subject seated with legs dangling and performed knee extension from lower-limb-dangling position to knee-extended position. Each SWDM exercise was repeated 15 times at 30 rep/min using a metronome. For each SWDM exercise, the peak amplitude during concentric contraction was measured in 12 trials, excluding the first 3 trials (where frequent EMG pattern variations were observed, probably due to the instability during initial periods of repetitive exercises), and the average value of 10 (excluding the highest and lowest values) was used as the 100% SWDM value. For mean dynamic method (MDM) and peak dynamic method (PDM), the RMS of EMG activity of propulsion was expressed as a percentage of the mean and the peak RMS of EMG activity of the intra-individual ensemble average,

$$X_{norm} = \frac{X - X_b}{X_{mean} - X_b} \tag{1}$$

and

$$X_{norm} = \frac{X - X_b}{X_{peak} - X_b}, \tag{2}$$

respectively, where X is the current value of the considered variable, X_{norm} is its normalized value, X_b is the baseline activity of RF muscle, X_{mean} and X_{peak} are the mean and maximum value observed along gait cycle.

As previously stated, the used normalization methods yield an output that is simply the ratio of the task EMG to the EMG used as the denominator in the normalization equation. As such, depending on the nature of the denominator, outputs from different normalization methods can differ in magnitude and pattern.

As depicted in Figure 3, different EMG normalization procedures lead to significant differences on the relative EMG amplitude developed during the activity assessed. Analyzing the normalized values of RF during propulsion it can be noted that this activity is extremely low when compared to the one obtained in maximal and submaximal contractions. However, this kind of normalization has a biological meaning since the values obtained during the activity are a percentage of the values obtained during maximal and submaximal contractions. The results show a higher magnitude of output from the MDM and PDM, which occurs as a result of using a smaller denominator in the normalization equation. Differences between these two methods are comprehensible taking into account the denominator values. Comparing MDM and PDM and the maximal and submaximal contraction methods, the first are more difficult to interpret as they are a percentage of the values obtained during the task. The RVC and SWDM normalization methods lead to relative values that are between the values obtained in the other methods and have more biological meaning than MDM and PDM.

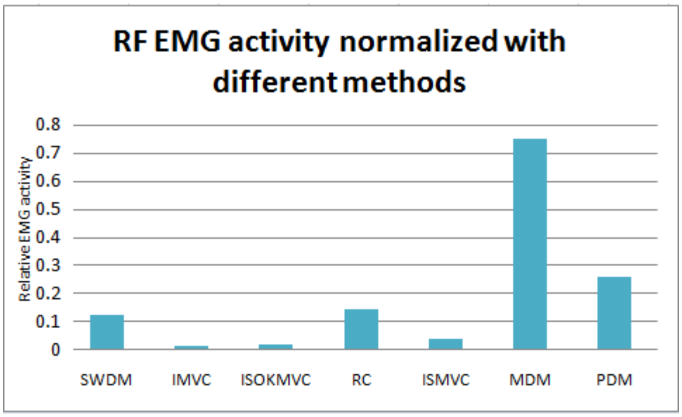

Figure 3: Relative EMG activity of RF obtained during gait propulsion at self-selected speed. Different normalization methods were used: segment weight dynamic movement (SWDM), isometric MVC (IMVC), isokinetic MVC (ISOKMVC), reference contraction (RC), isometric submaximal voluntary contraction (ISMVC), mean dynamic method (MDM) and peak dynamic method (PDM).

5. Effect of normalization method on inter-subject variability of the EMG signal

The use of normalization methods similar to the mean dynamic and peak dynamic methods has successfully reduced the inter-subject variability (Burden, et al., 2003; Winter & Yack, 1987a; Yang & Winter, 1984). In addition, there is strong evidence that using the dynamic mean normalization reduces the inter-subject variability in relation to other normalization methods (Allison, et al., 1993; Burden & Bartlett, 1999; Burden, et al., 2003; Knutson, et al., 1994; Yang & Winter, 1984) and the un-normalized EMG (Allison, et al., 1993; Burden & Bartlett, 1999; Yang & Winter, 1984). Thus, if researchers or clinicians wish to retain the homogeneity of task-specific EMG signal for a group of individuals, they should avoid use of the peak dynamic method and, in particular, the mean dynamic normalization methods, as suggested elsewhere (Allison, et al., 1993; Knutson, et al., 1994). According to (Burden, et al., 2003), normalization by mean dynamic method resulted in slightly more homogeneous pattern of gait EMG signal than the peak dynamic method. As to isokinetic maximal voluntary method, it should not be used in preference to the other methods, as is less reliable than un-normalized EMG signal or those normalized by the mean dynamic, peak dynamic or isometric maximal voluntary contraction methods (Burden, et al., 2003).

6. Ability of normalization method to detect changes in external force

According to (Allison, et al., 1993; Burden & Bartlett, 1999; Burden, et al., 2003), the isometric and isokinetic MVC methods reflect the increase in EMG that occurs in response to increments in external force. Unlike the MVC methods, the dynamic mean and dynamic peak normalization methods are not designed to provide the percentage of the maximal activation capacity of the muscle required to perform the isotonic contractions. Hence, it is unsurprising that the output of the mean dynamic normalization, and in particular the mean dynamic normalization methods, were unable to reflect the increase in EMG that occurred in response to the increase in force (Burden & Bartlett, 1999). This disagrees with the findings of (Allison, et al., 1993) stating that normalization methods using either the mean or the peak EMG from the ensemble average were able to distinguish between load and no-load conditions for the same muscle.

7. Conclusion

Different electromyography amplitude normalization methods have been described. We reviewed several studies that focus on comparing the different methods. Isometric and dynamic methods seem to be the most recommended. However, both present advantages and limitations, being important to understand clearly the purpose of the electromyographic study and the implications of the method adopted on the interpretation of the results attained. Table 1 summarizes the main topics discussed in this chapter concerning EMG signal normalization procedures and interpretation.

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Table 1: Advantages, disadvantages/limitations and interpretation of normalization methods.

Normalization method		Advantages	Disadvantages/Limitations	Data interpretation
Isometric method	*MVC method*	Perhaps the most powerful strategy for physiologic interpretation in healthy people.	○ Poor reliability. ○ Is affected by inertial effects at the onset of the test, patient fatigue, patient posture, synergistic contribution, patient motivation, pain, neuro-muscle-skeletal dysfunctions and neurologic conditions. ○ May not represent the maximum activation capacity in other lengths or under non-isometric conditions.	Represents the percentage of the maximum neural drive acquired while a subject performs an isometric MVC of the desired muscle. Any change in EMG amplitude indicates a true increase or decrease in the neural drive.
	Submaximal voluntary method	Resolves the instability of the EMG signal at near maximal levels.	○ Inter-subject coefficient of variation generally increases by using either 50% of the isometric MVC. ○ Values can be lower than the obtained during the activity. ○ Is affected by inertial effects at the onset of the test, patient fatigue, patient posture, synergistic contribution, patient motivation, pain, neuro-muscle-skeletal dysfunctions and neurologic conditions. ○ Does not represent a dynamic contraction.	Percentage of the maximum neural drive acquired while a subject performs an isometric submaximal voluntary contraction of the desired muscle. Any change in EMG amplitude indicates a true increase or decrease in the neural drive.
	RVC method	Helpful for clinical populations who are unable to attempt maximal efforts or who need a similar controlled task for interpreting repeated tests.	○ Is affected by inertial effects at the onset of the test, patient fatigue, patient posture, synergistic contribution, pain, neuro-muscle-skeletal dysfunctions and neurologic conditions. ○ Does not represent dynamic contraction.	Any change in normalized EMG amplitude indicates a true increase or decrease in the neural drive.
Isokinetic actions	*Isokinetic MVC method*	Has been used as a method to simulate with a higher degree of comparability muscle contractions obtained in dynamic activities.	○ Is less reliable than the other normalization methods.	Represents the percentage of the maximum neural drive acquired while a subject performs an isokinetic MVC of the desired muscle. Any change in EMG amplitude indicates a true increase or decrease in the neural drive.

Normalization method		Advantages	Disadvantages/Limitations	Data interpretation
Dynamic muscle actions	*Mean dynamic method*	Reduces the inter-subject variability in relation to other normalization methods. Helpful for clinical populations that are unable to attempt maximal efforts.	○ Tends to produce a normal EMG template for a particular task and, therefore, may remove the true biological variation within a group. ○ It may be more susceptible to systems with a low signal to noise ratio or represent baseline noise in movements that cause very phasic activation. ○ It does not give an indication of what this activity level means with respect to the muscle's ability to activate.	Represents a percentage of the average of both quiet and active periods during the activity.
	Peak dynamic method	Reduces the inter-subject variability in relation to other normalization methods. Helpful for clinical populations that are unable to attempt maximal efforts.	○ Tends to produce a normal EMG template for a particular task and, therefore, may remove the true biological variation within a group. ○ It does not give an indication of what this activity level means with respect to the muscle's ability to activate.	Indicates at what periods during the activity the muscle is most active.

PART B – *ARTICLE II*

Effect of gait speed on muscle activity patterns and magnitude during stance

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Motor Control (2012); 16:480-492

ABSTRACT

Purpose: This study aims to assess the influence of gait speed (manipulated through cadence) on muscle activity patterns and activation degree during stance. *Methods:* Thirty-five healthy individuals participated in this study. Surface electromyographic activity from the gastrocnemius medialis (GM), gluteus maximus (GMax), biceps femoris (BF) and rectus femoris (RF) was acquired with subjects walking at three different speeds. *Results:* Speed influenced: (1) relative motor activity patterns at heel strike, midstance-propulsion transition and propulsion; (2) the activity level of RF, GMax, GM and BF, in decreasing order, with higher activity at the fastest and slowest speeds. *Conclusions:* In general, muscle activity was higher at the fastest and slowest speeds than at the self-selected speed and only the activity of the main actions in each subphase remained stable. These findings suggest that gait speeds different from the self-selected speed influence not only activity levels but also relative muscle activity patterns. As a result, caution is advised when choosing standard speeds in gait studies, as this can lead to increased variability in relative muscle activity patterns.

***Keywords*:** electromyography; gait; muscle function; motor control; motor behavior; motion analysis.

1. INTRODUCTION

It is well known that there are some factors which account for metabolic energy expenditure during gait, such as the need to redirect the center of mass (COM) (Kuo, et al., 2005), body weight support, leg movement or stability control (Liu, et al., 2006; Neptune, et al., 2001; Zajac, et al., 2003). This metabolic energy needed to walk is explained by the mechanical power generated by muscles (Woledge et al., 1985), whose activity patterns and intensity of activation change with speed (Crowe et al., 1993, 1995; den Otter, et al., 2004; Ivanenko, et al., 2006) to control the accelerating and decelerating forces of individual body segments needed to establish safe forward progression (Yang & Winter, 1985). Muscle activity during locomotion occurs in bursts that change in both amplitude and duration as a function of locomotion speed (den Otter, et al., 2004; Ivanenko, et al., 2004; Nilsson et al., 1985). According to Cappellini et al, 2006, changes in walking speed are associated mainly with modifications in the intensity of muscle activation and only minor changes are observed in their relative timings during stance (Cappellini, et al., 2006;

den Otter, et al., 2004). Also the temporal EMG profiles of functionally related muscles can show considerable similarity at different cadences (Davis & Vaughan, 1993; Yang & Winter, 1985).

The simple model of gait described in (Kuo, 1999) predicts that collision costs increase with step length and frequency, accounting for a significant part of the energy consumed during walking. The amplitude of muscle activity increases with walking speed because of the need for larger muscular force output. This normal positive relationship may be challenged if walking speed is markedly reduced, due to changes in the underlying locomotor task demands. Extreme reductions in walking speed will prolong substantially the time spent in double support, and one may expect a switch from locomotor to merely postural muscular synergies. Also, the larger horizontal excursions of the COM associated with slow walking may necessitate more explicit muscular efforts to maintain frontal plane balance during walking (Bauby & Kuo, 2000). Moreover, at self-selected walking speeds energy expenditure is diminished, as the gait system is adapted to execute walking at the usual speed (Masani et al., 2002).

In general, muscles are most active around the time of foot contact (Singh, 1970), as there is a distribution of hip and knee extensor muscle forces in early stance and ankle plantar flexor and rectus femoris forces in late stance to provide support and forward propulsion (Liu, et al., 2006). However, how the muscle contributions change with walking speed is not well understood. Intuitively, walking at faster speeds would require increased activity from muscles contributing to forward propulsion. However, fast walking speeds are associated with longer stride lengths, which may require increased activity from those muscles contributing to vertical support, as there is an increased vertical excursion of the body's COM (Orendurff et al., 2004). On the other hand, walking at slower speeds may be mechanically less efficient and less conducive to storage and recovery of elastic energy in the musculotendon complex (Neptune et al., 2008).

Most authors studying the influence of speed on gait muscle activity have used treadmills (den Otter, et al., 2004; Masani, et al., 2002; Nilsson, et al., 1985; van Hedel, et al., 2006), but there are some differences between treadmill and overground walking. During treadmill walking, increases in the hip range of motion, maximum hip flexion, joint angle and cadence occur, while the stance time decreases (Alton et al., 1998). Knee kinematics appear to be comparable (Matsas et al., 2000), while vertical ground reaction forces show similar patterns, but differ slightly in force magnitude during mid and late stance (White et al., 1998). Leg muscle EMG activity is somewhat smaller in overground versus treadmill walking (Arsenault, Winter, et al., 1986b; Murray et al., 1985). The effect of speed on overground walking has already been studied, but only addressing the muscle recruitment timing with the individuals walking at the same standard speed (Murray et al., 1984). However, as already mentioned, the gait system is adapted to walking at different speeds, and therefore it is important to study the influence of different gait speeds on muscle recruitment patterns compared to the self-selected speed adopted by each subject.

Therefore, the purpose of this study is to examine how muscle activation depends on locomotion speed during overground walking. Specifically, this study aims to address the influence of speed variation, based on self-selected speed, on intensity and muscle activity patterns during stance phase of walking. To achieve this goal, we tested the following hypotheses: (1) there will be differences in muscle activity level between locomotion at self-selected speed and at slower and faster speeds, (2) there will be no differences in relative muscle activity patterns between the different speeds. The findings should provide information on the neuromuscular strategies related to locomotion at different speeds.

2. METHODS

2.1 Subjects

Thirty-five healthy female subjects were recruited (age = 21.6±3.17 years, height = 1.65±0.045 m, body weight = 58.8±7.72 kg, left Q angle = 14.57±0.85 degrees; right Q angle = 14.7±0.96º degrees; mean±S.D.); possible candidates with at least one of the following criteria were excluded: history of recent osteoarticular or musculotendon injury of the lower limb or signs of neurological dysfunction which could affect lower limb motor performance; history of lower limb surgery; lower limb anatomical deformities, Q angle below 14º or above 17º (Nguyen et al., 2009), due to the possibility that biomechanical changes resulting from abnormal alignment might influence joint loads, mechanical efficiency of muscles, and proprioceptive orientation and feedback from the hip and knee, resulting in altered musculoskeletal function and control of lower extremities (Shultz et al., 2006). All subjects were right-leg dominant.

The study was conducted according to the ethical norms of the Institutions involved and conformed to the Declaration of Helsinki, 1964. Informed consent was obtained from all participants.

2.2 Instrumentation

Ground reaction force (GRF) values were obtained from a force plate, model FP4060-10 from Bertec Corporation (USA), connected to a Bertec AM 6300 amplifier, with default gains and a 1000 Hz sampling rate. The amplifier was connected to a Biopac 16 bit analogical-digital (A/D) converter from Biopac Systems, Inc. (USA).

According to (Neptune, Zajac, et al., 2004), the muscle mechanical (and most likely metabolic) energetic cost is dominated not only by the need to redirect the COM in double support but also by the need to raise the COM in single support. The authors showed that the muscles responsible for most of the work on the COM were the gluteus maximus (GMax), hamstrings, rectus femoris (RF), gastrocnemius medialis (GM), soleus and vasti muscles. Hence, we selected a representative muscle for each subphase. As such, the electromyographic activity (EMGa) of GMax, BF, RF and GM muscles was monitored using the model MP 100 Workstation from Biopac Systems, Inc. (USA), with a sampling rate of 1000 Hz and an amplified band-pass filter between 10-500 Hz (common mode rejection ratio (CMRR)>110 dB, gain=1000) and analog-to-digital converted (12 bit). Data were collected using steel surface electrodes, model TSD150 from Biopac Systems, Inc. (USA), bipolar configuration, with a 11.4 mm contact area and an inter-electrode distance of 20 mm, and a ground electrode. Skin impedance was measured with an Electrode Impedance Checker (Noraxon USA, Inc.)

Gait timing was measured using a photovoltaic system, model IRD-T175 from Brower Timing Systems (USA), and a metronome, model TempoPerfect Metronome Software from NCH Software (USA), was used to help subjects control gait cadence.

The signals acquired were processed with Acqknowledge version 3.9 for MP150 system (Biopac Systems, Inc. (USA)).

2.3 Procedures

2.3.1 Self-selected speed definition

All subjects walked along a 10 m walkway (Chen et al., 1997; Whitle, 2007). They were instructed to walk along a force plate located in the middle of the walkway and to keep walking past the reference point without stopping. Self-selected cadence was recorded for each subject, from which two different cadences were defined - one 25% higher and the other 25% lower - and each subject was studied while walking at these three cadences. Average walking speed was assessed by measuring the time interval between two infrared beams at either end of the walkway, 8 m apart (Whitle, 2007). To diminish possible speed variations resulting from acceleration and deceleration, subjects walked a distance greater than the distance being monitored.

2.3.2 Skin and instrument preparation

The subjects' lower limb skin surfaces were prepared to reduce electrical resistance to less than 5000 Ω (Basmajian & De Luca, 1985) by (1) shaving the skin surface of the muscle belly area; (2) removing dead cells with alcohol; and (3) removing non-conductor elements between electrode and muscle with an abrasive pad (Hermens et al., 2000).

Measurement electrodes were placed at GM, BF, RF and GMax mid-belly according to anatomical references and fixed with adhesive tape (Basmajian & De Luca, 1985; Freriks et al., 1999). Measurements began 5 minutes after electrode placement as evidence suggests that there is a reduction of 20 to 30% in impedance values during the first 5 minutes after electrode placement (Vredenbregt & Rau, 1973).

2.3.3 Measurement

Subjects were required to walk at three different speeds (based on self selected, fast and slow cadence) after adequate practice (10-15 trials) to reach a steady pace in which the dominant foot stepped onto the plate. The subjects were barefoot and were asked to look straight ahead and walk, as naturally as possible, for a minimum of 8 steps (James et al., 2007). They performed each speed three times, with metronome feedback. Only one foot at a time had full contact with the plate, and there was no extra load of any kind on the plate. Measurements were randomised to reduce the order effect, which can be caused by fatigue and previous muscle activation, and all procedures and verbal commands were given equally to all subjects.

The EMGa for each muscle was collected by a four-channel unit at 1000 Hz. The signals were pre-amplified at the electrode site and then fed into a differential amplifier with an adjustable gain setting (12-500 Hz; CMRR: 95 dB at 60 Hz and input impedance of 100 MΩ). Raw energy signals were digitised and stored on computer disks for subsequent analysis by the Acqknowledge software. Then, the signal was filtered (20-500 Hz) and the root mean square (RMS) values for each muscle were calculated for each subphase (Basmajian & De Luca, 1985; Medved, 2001). They were also normalised according to the peak of the subject ensemble average (Yang & Winter, 1984). GRF data were filtered using a low-pass filter with a 20 Hz cutoff frequency and normalised according to weight (Mullineaux et al., 2006). EMG and GRF data were collected on the same A/D board and the subjects' weight was measured during static posture on the plate.

The stance phase was defined as the interval during which the vertical reaction force exceeded 7% of body weight. This phase was then segmented into four subphases recognised in the trace of the vertical component of GRF. The time from heel strike to the first main peak of force, due to loading, was designated in this study as heel strike (HS). Midstance (MS) spans the interval between the two main peaks in the vertical force. The time between the first main peak of force and the local minimum during midstance was designated as transition between heel strike and midstance (HS-MS). The time between the local minimum during midstance and the second main peak was designated as transition between midstance and propulsion (MS-P). Late stance matches the final push-off phase of the ipsilateral limb; it lasts from the second peak of the vertical force to toe-off and was designated as propulsion (P).

2.3.4 Statistics

Data were analysed with the Statistical Package Social Science software version 16.0 from SPSS Inc. (USA) using a significance level of $\alpha<0.01$.

As speed was not controlled directly but through step cadence, the ANOVA (Analysis of Variance) test was used to analyse speed differences. The Friedman and Wilcoxon tests were used to compare muscle activation levels at different stance subphases and speeds and to analyse the influence of speed on muscle activity patterns.

3. RESULTS

3.1 Gait speeds adopted

There were statistically significant differences between the walking speeds adopted (Table 1), and as such our results can be further discussed under this premise.

3.2 GM, GMax, BF and RF activity patterns during stance at different speeds

At all speeds and stance subphases there were significant differences in relative muscle activity recruitment for GM, GMax, BF and RF ($p<0.0001$).

Table 1: Registration of mean, standard deviation, maximum and minimum values for the three speeds adopted and p values obtained from the repeated measures ANOVA to compare all speeds.

Speed (m/s)	N	Mean±Standard deviation	p values
Slow	35	1.32±0.29	
Self-selected		1.56±0.36	<0.0001
Fast		1.81±0.43	

At HS, GMax exhibited the highest activity at all speeds, followed by RF and then by BF and GM (fast: GM-Gmax/BF/RF, $p<0.0001$; BF/RF-GMax, $p=0.001$; self-selected: GM-BF/RF/GMax, $p<0.0001$; GMax-BF/RF, $p=0.004$; slow: BF-RF/GMax, $p=0.001$; BF-RF, $p=0.005$; GMax-GM/RF, $p<0.0001$; GM-RF, $p<0.0001$).

For values obtained during HS-MS, a similar pattern was observed at all speeds, with GMax and GM exhibiting the highest activity, followed by RF and BF, although at the fastest walking speed there were no significant differences between RF and Gmax and GM (fast: RF-BF, $p=0.003$; BF-GMax, $p=0.001$; GM-BF, $p<0.0001$; self-selected: RF-GMax, $p=0.005$; GMax-BF, $p=0.001$; GM-BF, $p<0.0001$; slow: RF-BF, $p=0.002$; GMax-BF/RF, $p<0.0001$; GM-BF, $p<0.0001$).

During MS-P, at the fastest speed the GM exhibited the highest activity, followed by GMax and RF, and lastly by BF (RF-BF, $p=0.002$; GM-GMax, $p=0.001$; RF-GM, $p<0.0001$; GMax/GM-BF, $p<0.0001$). At self-selected speed the GM showed the highest activity, when compared to RF and BF, and GMax exhibited the same activity level as GM (RF-GM, $p=0.001$; GMax-BF, $p=0.003$; GM-BF, $p<0.0001$). At the slowest speed GM and GMax showed the highest activity, followed by RF and then by BF (RF-BF, $p=0.005$; GMax-BF, $p<0.0001$; GMax-RF, $p=0.001$; GM-RF/BF, $p<0.0001$).

During P, the RF presented the highest activity at the fastest and slowest speeds, followed by BF and GMax, and finally by GM, while at self-selected speed the highest activity was exhibited by RF and BF, followed by GMax and GM, these last two muscles showing no significant differences (fast: GMax-GM, $p=0.001$; BF/GMax/GM-RF, $p<0.0001$; GM-BF, $p=0.002$; self-selected: BF-GMax, $p=0.001$; GMax/GM-RF, $p<0.0001$; BF-GM, $p<0.0001$; slow: GM-BF, $p=0.001$; GMax-GM, $p=0.007$; GMax/GM-RF, $p<0.0001$; RF-BF, $p=0.008$).

3.3 Speed influence on muscle recruitment level in each stance subphase

Figures 1-4 show speed influence on muscle activity in the different stance subphases. BF activity showed speed-related changes only during HS, decreasing at self-selected speed ($p=0.001$). RF activity is most prominent at the fastest and slowest speeds ($p<0.0001$) at HS, while in the other subphases it exhibits higher activity at the fastest speed ((HS-MS, $p<0.0001$ (fast-slow) and $p=0.001$ (self-selected-fast); MS-P, $p<0.0001$ (fast-slow) and $p=0.002$ (self-selected-fast); P, $p=0.002$)) GMax activity was higher at the fastest and slowest speeds than at self-selected speed (HS, $p=0.002$; MS-P, $p<0.0001$), except during HS-MS and P. During HS-MS, GMax showed no speed-related changes, and during P the highest GMax activity occurred at the fastest walking speed ($p<0.0001$). GM activity increased with speed at the fastest and slowest walking speeds (in decreasing order) only during MS-P ($p<0.0001$).

A comparison of each muscle activity during the different stance subphases shows statistically significant differences, as shown in Figures 1-4. At the fastest speed, GM showed differences in all subphases ($p<0.0001$). At all speeds this muscle exhibited the highest activity during MS-P, followed by HS-MS, HS, and finally by P. GMax presented the same pattern at all speeds, the highest activity occurring during HS, followed by HS-MS and MS-P, and finally by P ((HS vs HS-MS, $p<0.0001$; MS-P vs P, $p=0.001$ (fast) and $p<0.0001$ (self-selected and slow); HS vs MS-P, $p<0.0001$ (fast and slow) and $p=0.001$ (self-selected); HS-MS vs P, $p=0.005$ (fast) and $p<0.0001$ (self-selected and slow); HS-P, $p=0.001$ (fast) and $p<0.0001$ (self-selected and slow)). RF, at all speeds, exhibited the highest activity during P, followed by HS, with no difference at the other subphases ((HS vs HS-MS, $p<0.0001$; MS-P vs P, $p<0.0001$ (self-selected); HS vs MS-P, $p<0.0001$; HS-MS vs P, $p=0.006$ (self-selected); HS vs P, $p=0.001$ (fast), $p=0.002$ (self-selected) and $p=0.004$ (fast)). BF contribution was higher during HS for the fastest and slowest speeds. At self-selected speed its activity was higher at HS and P ((HS vs HS-MS, $p<0.0001$; MS-P vs P, $p=0.0077$ (fast), $p=0.002$ (self-selected) and $p=0.001$ (slow); HS vs MS-P, $p<0.0001$; HS-MS vs P, $p=0.0031$ (fast), p=(0.002 (self-selected) and $p=0.001$ (slow); HS vs P, $p=0.0024$ (fast)).

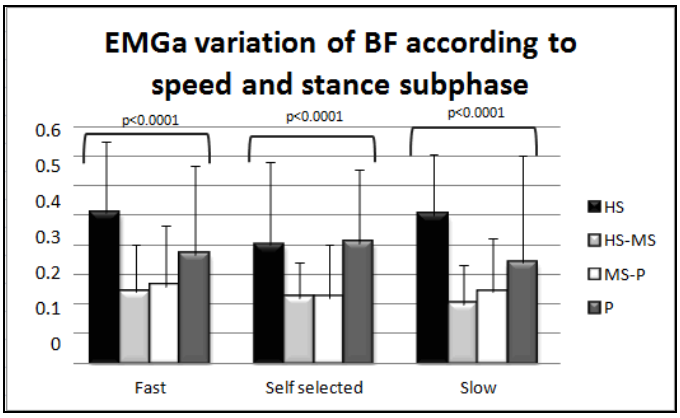

Figure 1: Biceps femoris (BF) activity in the different stance subphases at the three walking speeds. Friedman test p values obtained from comparisons of BF activity among all stance subphases for each speed are presented.

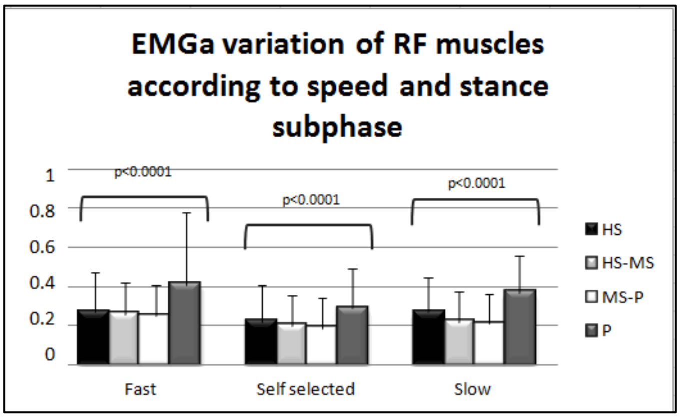

Figure 2: Rectus femoris (RF) activity in the different stance subphases at the three walking speeds. Friedman test p values obtained from comparisons of RF activity among all stance subphases for each speed are presented.

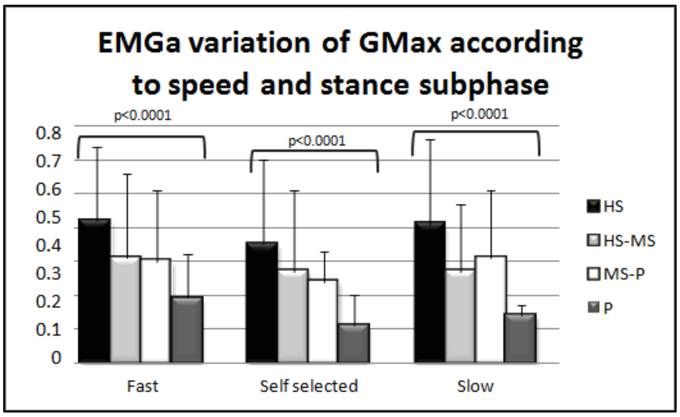

Figure 3: Gluteus maximus (GMax) activity in the different stance subphases at the three walking speeds. Friedman test p values obtained from comparisons of GMax activity among all stance subphases for each speed are presented.

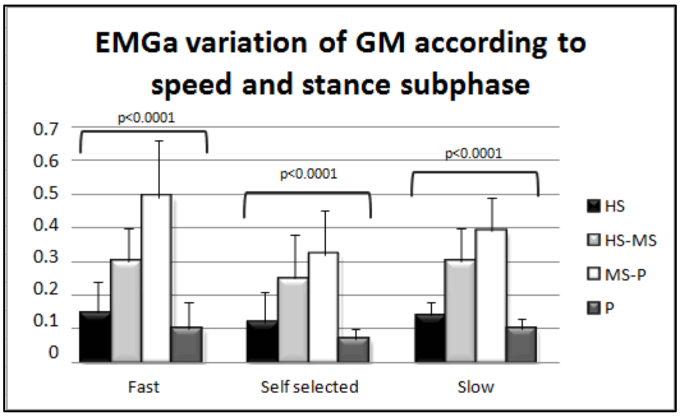

Figure 4: Gastrocnemius medialis (GM) activity in the different stance subphases at the three walking speeds. Friedman test p values obtained from comparisons of GM activity among all stance subphases for each speed are presented.

4. DISCUSSION

4.1 Influence of gait speed on muscle activation recruitment patterns during stance

During HS, GMax exhibited the highest activity, which illustrates its importance during this subphase, as the passive support of the bones alone is not enough to prevent collapse. As a stance-side muscle, GMax plays an important role in the body's fore-aft deceleration during the first half of stance (Liu, et al., 2006). The lowest activity of this muscle at self-selected speed could be explained by the fact that total negative work is reduced during loading response at the self-selected speed and increases at faster and slower speeds, as a result of changes in stride length and activation-deactivation dynamics, which limit the rate at which the muscle force can deactivate (Neptune, et al., 2001; Neptune, et al., 2008). The second highest activity was exhibited by RF, although according to (Nene et al., 2004) during swing to stand transition RF activity is probably due to crosstalk, which is consistent with the observation that the vasti muscles are the most active group (Liu, et al., 2006; Neptune, Kautz, et al., 2004; Zajac, et al., 2003). The importance of hamstrings in preventing knee hyperextension during late swing has been mentioned, suggesting the importance of this muscle on loading response (Whitle, 2007). The results of this study demonstrate a significant activity of this muscle during HS. The lowest activity was exhibited by GM, whose activity was not affected by speed changes. This can be explained by GM role in this subphase, as plantar flexors begin to support the trunk in early single-leg stance, because of their individual contributions to the hip inter-segmental force, which accelerates the trunk upwards before MS (Neptune, et al., 2001).

During HS-MS, the higher activity of GMax and GM is supported by the importance of hip extensors in the development of positive work by concentric activity and the importance of GM contribution to trunk support, acting almost isometrically, and in the execution of negative work, when the tibia rotates over the foot (Neptune, et al., 2001; Norkin & Levangie, 1992). A similar pattern for GMax and GM at all speeds seems to be explained by the role of these muscles in body support during this subphase (Neptune, et al., 2001). It is important to note that at the fastest walking speed, RF activity matched GM and GMax levels which is consistent with RF activity being higher at the fastest speed. In fact, during MS knee extensors execute negative work, acting eccentrically to control knee flexion (Norkin & Levangie, 1992).

The preponderance of GM during MS-P at all speeds seems to suggest its importance in forward propulsion (Liu, et al., 2006). It is important to note that GM activity was only affected by speed during MS-P, which is supported by findings obtained by (Neptune & Sasaki, 2005) demonstrating that the ability of plantar flexors to produce force during propulsion is clearly affected as walking speed increases. As plantar flexors have been considered important contributors to support forward progression and initiation of swing during walking (Liu, et al., 2006; Neptune, et al., 2001; Zajac, et al., 2003), it might be necessary to increase the activity of other muscles to compensate for the decrease in plantar flexor activity. This can support the proximity of GMax to GM activity level. At the slowest speed, this can be explained by the temporarily lower stability at slow speeds than at fast speed (England & Granata, 2007). RF and GM were the only muscles affected by speed, exhibiting higher activity at the fastest speed, as these two muscles work in synergy to provide trunk forward propulsion in walking (Jonkers et al., 2003; Neptune, Kautz, et al., 2004; Neptune, et al., 2008; Zajac, et al., 2003).

During P, the RF showed the highest activity at all speeds, which may be explained by its contribution to trunk forward propulsion in late stance (Neptune, et al., 2001). This muscle was the most affected by speed, showing higher muscle activity magnitude at faster speeds. According to (Chen, et al., 1997) the human body tends to use the quadriceps muscle first to increase walking speed.

4.2 Influence of gait speed on muscle contribution in stance

The higher activity of GM during MS-P at all speeds seems to be consistent with other studies (Liu, et al., 2006; Neptune, et al., 2008). Energy generation by plantar flexors during late stance corresponds to the greater work executed in the gait cycle and accounts for vertical and horizontal accelerations (Winter, 1991). It is important to note that during HS-MS GM exhibited the second highest activity at all speeds, corroborating dynamic simulations that demonstrate that the sum of the effects of plantar flexors during MS guarantees body support, allowing a consistent forward movement and thus preventing the collapse of the member (Neptune, et al., 2001; Simon et al., 1978). In this study, the contribution of GM to P was not predominant, which supports the statement that in this subphase there is higher contribution from the soleus muscle to increase speed (Zajac, et al., 2003). RF exhibited the highest activity during P at all speeds, promoting trunk forward acceleration in synergy with the soleus muscle (Neptune, Kautz, et al., 2004). During this subphase RF acts as the antagonist of the gastrocnemius, supporting the low GM activity observed in this study. The higher activity of GMax at HS at all speeds is consistent with other studies (Neptune, et al., 2008), as already mentioned.

The results of this study corroborate the idea that the fundamental regions of muscle activity remain relatively stable during the gait cycle (den Otter, et al., 2004; Hof, et al., 2002).

4.3 Influence of gait speed on muscle recruitment level

A global analysis of the results shows that muscles controlling the most proximal joints are more affected by speed changes than the more distal ones, as there was more variation in GMax, RF and BF than in GM. This is explained by a transition between work generated at the ankle, knee and hip (Chen, et al., 1997), suggesting a transfer of work to larger muscle groups when walking at faster speeds, which would allow muscles to work at a lower percentage of their maximum capacity and therefore optimise energy consumption during gait.

These results also show that fast and slow speeds are associated with increased muscle activity. Bipedal walking analysis shows that there is a biomechanical resonance associated with the inverted pendulum-like behaviour of the skeletal structure and with muscle stiffness (Holt et al., 1990), which may contribute to stability in normal walking (McGeer, 1990). Walking speeds not corresponding to this resonance frequency value require more neuromuscular active control to maintain a periodic stable movement (Ralston, 1958). In other words, faster walking speeds increase the segmental momentum, thereby requiring greater effort from the neuro-controller to attenuate kinematic disturbances. Short stride durations limit the allowable time for neuromuscular corrections to compensate for mechanical disturbances or controller errors. Slow walking speeds require active control that is out-of-phase with movement in order to slow the natural dynamics of the passive system (Cavagna & Kaneko, 1977). Mean walking speeds obtained in this study range from 1.32 to 1.81 m/s. It is argued that, for a moderate walking speed (0.5 to 1.5 m/s), costs in the swing phase are reduced; the metabolic cost is explained by muscle force generation during the stance phase (Winter, 1991). As fast speeds used in this study exceeded the 1.5 m/s indicated it would be relevant to execute the same analysis during the swing phase.

PART B – *ARTICLE III*

Analysis of ground reaction force and electromyographic activity of the gastrocnemius muscle during double support

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ABSTRACT

Purpose: Mechanisms associated with energy expenditure during gait have been extensively researched and studied. According to the double-inverted pendulum model energy expenditure is higher during double support, as lower limbs need to work to redirect the CoM velocity. This study looks into how the ground reaction force (GRF) of one limb affects the muscle activity required by the medial gastrocnemius (MG) of the contra-lateral limb during step-to-step transition. *Methods:* Thirty-five subjects were monitored as to the MG electromyographic activity (EMGa) of one limb and the GRF of the contra-lateral limb during double support. *Results:* After determination of the Pearson correlation coefficient (r), a moderate correlation was observed between the MG EMGa of the dominant leg and the vertical (F_z) and anteroposterior (F_y) components of GRF of the non-dominant leg ($r=0.797$, $p<0.0001$; $r=-0.807$, $p<0.0001$) and a weak and moderate correlation was observed between the MG EMGa of the non-dominant leg and the F_z and F_y of the dominant leg, respectively ($r=0.442$, $p=0.018$; $r=-0.684$ $p<0.0001$). *Conclusions:* The results obtained suggest that during double support, GRF is associated with the EMGa of the contra-lateral MG and that there is an increased dependence between the GRF of the non-dominant leg and the EMGa of the dominant MG.

Keywords: Double-inverted pendulum model; Double support phase; Ground reaction force; Ankle plantar flexor activity; Electromyography.

1. INTRODUCTION

Several models have been suggested to describe human gait mechanisms (Cavagna, et al., 1977; Cavagna & Margaria, 1966; Donelan, et al., 2002b; Kuo, et al., 2005, 2007; Saunders, et al., 1953; Waters & Mulroy, 1999). Although neural and mechanical systems, including musculoskeletal dynamics, supra-spinal and afferent modulation, work together to minimise energy expenditure (Arechavaleta, et al., 2008; Borghese, et al., 1996), it is widely accepted that the recovery of mechanical energy during gait is incomplete, even though muscles work to compensate energy loss (Griffin, et al., 2003; Kuo, et al., 2005, 2007; Yakovenko et al., 2002). Understanding how individual muscles contribute to specific tasks can provide important insights into neuromuscular control and walking mechanics. This understanding can also aid in developing improved prosthetic, orthotic and other assistive devices to mitigate neuromuscular impairments and designing more effective rehabilitation strategies.

According to the double-inverted pendulum model (Donelan, et al., 2002a; Kuo, et al., 2007) a major energy loss in walking is due to step-to-step transitions (Donelan, et al., 2002a), which occur mainly during double support as the two leg forces need to redirect the centre of mass (CoM) velocity from a downward and forward direction to an upward and forward direction. The leading leg strikes the ground, performing negative work on the CoM and the energy lost may be restored through positive work by the trailing leg. The double-inverted pendulum model predicts that restoring mechanical work is done by the trailing leg at the instant of heel strike through a powerful plantar flexion (Kuo, 1999, 2002; Kuo, et al., 2005). Based on these assumptions, a relation between ankle plantar flexors muscle activity and heel strike force would be expected, as these muscles play a major role in propulsion in late stance during unimpaired walking (Liu, et al., 2006; Neptune, Kautz, et al., 2004) and are important to provide body support (Neptune, et al., 2001; Nguyen, et al., 2009; Shultz, et al., 2006), matching the second peak of the vertical ground reaction force (Winter, 1983). Considering predictions of the double-inverted pendulum model, impact forces can demonstrate the role of the gastrocnemius muscle: it contributes to push-off and limits heel strike. According to Doets et al., 2009, the larger the heel strike cost in the leading leg during heel strike, the higher the metabolic cost of walking, i.e., the energy dissipated during step-to-step transition explains 29% of the variance in the metabolic energy cost of walking.

Neurophysiologically, the regulation of human walking requires a close coordination of muscle activation between the two legs which seems to be achieved by a flexible neuronal coupling at spinal level (for reviews, see, for example, (Dietz, 1992; Dietz, 2002)). During gait, a perturbation of one leg evokes a purposeful bilateral response pattern, with a similar onset latency on both sides, which is thought to be mediated at spinal level under supraspinal control (Berger et al., 1984; Dietz, 1992; Dietz & Berger, 1984; Dietz et al., 1986). Also, there is evidence of bilateral interlimb coordination in homonymous muscle groups in the human, as each limb affects the strength of muscle activation and the time-space behavior of the other (Stubbs et al., 2009).

Many different groups of afferents from flexor and extensor muscles can influence the locomotor pattern. Most attention has focused on the action of group I afferents from ankle extensors, which contributes to 30-60% of ankle extensor activity (Sinkjaer, et al., 1996). Feedback-mediated reinforcement of ankle extensor muscle activity contributes, together with supraspinal drive and possibly spinal drive (i.e., central pattern generator), to propel the body forward. During the stance phase of the human step cycle, the ankle undergoes a natural dorsiflexion that stretches the soleus muscle (Sinkjaer, et al., 1996). In (Capaday & Stein, 1987), it has been reported that the Hoffman reflex is relatively low at the time of heel contact, increases progressively during the stance phase, and reaches its maximum amplitude in the late stance phase. However, ankle extensor muscle velocity during normal walking is slow (Fukunaga et al., 2001) suggesting that group Ia afferent pathways may not be effectively recruited during normal walking. In fact, there is a growing body of evidence suggesting that load information provided by group Ib afferents, arising from force-sensitive Golgi tendon organs, contributes substantially to ongoing muscle activity. In addition, it has been suggested that tendon organ feedback via an excitatory group Ib pathway contributes to the late stance enhancement of the soleus muscle activity (Grey, et al., 2007). In (Pearson & Collins, 1993), it has been found that electrical stimulation of an extensor nerve at group I during walking in the cat substantially increased ongoing activity in other extensor muscles (medial and lateral gastrocnemius).

Taking into account the above and that ground reaction force (GRF) is regarded as a representative measurement of gait, because it is the external force involved in walking and affects the acceleration of the body's centre of mass (Winter, et al., 1990), the main purpose of this study is to investigate how much a relatively simple measure such as the heel strike force can explain plantar flexor muscle activity during propulsion of the contra-lateral leg. Such knowledge can, for instance, be used to evaluate patients' gait and the efficacy of prosthetic and orthotic devices. The results obtained will also contribute to understand the mechanisms involved in the double support phase of walking, specifically the function of plantar flexors.

2. Methods

2.1 Subjects

Thirty-five healthy female subjects were tested (age = 19.7±1.3 years, height = 1.65±0.045 m, body weight = 56±5.4 kg, non-dominant Q angle = 14.57±0.85 degrees; dominant Q angle = 14.7±0.96 degrees; mean±S.D.). Individuals not matching at least one of the following criteria were excluded: history of recent osteoarticular or musculotendon injury of the lower limb or signs of neurological dysfunction which could affect lower limb motor performance; history of lower limb surgery; lower limb anatomical deformities, Q angle below 14º or above 17º (Nguyen, et al., 2009). Biomechanical changes resulting from abnormal alignment may influence joint loads, the mechanical efficiency of muscles, and proprioceptive orientation and feedback from the hip and knee, resulting in altered neuromuscular function and control of lower limbs (Shultz, et al., 2006). All subjects were right-leg dominant.

The study conformed to the ethical norms of the Institutions involved and to the Declaration of Helsinki, dated 1964. Informed consent was obtained from all participants.

2.2 Instrumentation

Vertical (F_z), anteroposterior (F_y) and mediolateral (F_x) GRF values were obtained from a force plate, model FP4060-10, from Bertec Corporation (USA), connected to a Bertec AM 6300 amplifier, with default gains and a 1000 Hz sampling rate. The amplifier was connected to a Biopac 16-bit analogical-digital converter, from BIOPAC Systems, Inc. (USA). The floor and the underlying structure were rigid and flat to minimise any vibrations. Also, the top of the force plate was at the floor level, which was obtained by having a raised walkway. To avoid measurement errors, a gap of 1-2 mm was left between the force plate and the surrounding floor. Reliability of measurements of GRF magnitude has an intraclass correlation coefficient (ICC) of 0.88 (Hanke & Rogers, 1992). Medial gastrocnemius (MG) electromyographic activity (EMGa) was monitored using Biopac Systems, Inc – MP 150 Workstation; TD150B steel electrodes were used with bipolar configuration, 20 mm between detection surfaces (centre to centre) and a reference electrode. At preferred walking speeds the total energy of the mean EMG value averaged across the gait cycle has been shown to be highly consistent (Arsenault, Winter, Marteniuk, et al., 1986). Gait timing was measured by the Brower Timing system (IRD-T175, Utah, USA), which presents a sensitivity of 0.01 seconds. For each subject, the time interval measured was used to calculate the mean speed of walking in each trial. Two pressure transducers (TSD111, BIOPAC Systems, Inc.) were used to access gait cycles of the trailing leg. Q angle measurement was performed with a Baseline universal goniometer. Intra-rater reliability for measuring Q-angle in the supine position presents ICC values of 0.94 (Ferro, 2010). Skin impedance was measured with an Electrode Impedance Checker (Noraxon USA, Inc.). Signals obtained from the force plate, the electromyography and the pressure transducer were processed with Acqknowledge, version 3.8, from BIOPAC Systems, Inc.

2.3 Procedures

Subjects were asked to perform two series of three trials of walking at self-selected speed, as three strides of EMG data per subject provide reliable information (Arsenault, et al., 1986). The EMGa of the MG muscle of one leg (the trailing leg) and the GRF of the contra-lateral leg (the leading leg) were collected. In the first series, the EMGa of the dominant limb during propulsion and the GRF of the non-dominant limb at heel strike were monitored. In the second series, we collected the EMG signal of the non-dominant limb during propulsion and the GRF of the dominant limb at heel strike. Measurements were randomised to prevent possible influence from order or learning effects. The lateral gastrocnemius was not measured as it has been documented that EMG patterns of both heads of the gastrocnemius are similar in terms of the timing of activation during walking (Sutherland et al., 1980; Winter, 1983). In addition, it appears that the MG plays an important role in forward propulsion, whereas the soleus does not (Gottschall & Kram, 2003).

2.3.1 Skin and instrument preparation

Skin surface of the subjects' lower limbs was prepared to reduce electrical resistance to less than 5000 Ω (Basmajian & De Luca, 1985): shaving of the MG area; removal of dead skin cells with alcohol; removal of non-conductor elements with abrasive pad (Turker, 1993). Measurement electrodes were placed at the MG centre, according to (Hermens, et al., 2000), and fixed with adhesive tape, to prevent displacement and to guarantee homogeneous and constant pressure. The reference electrode was placed on the patella. Between electrodes positioning and the beginning of measurements we set an interval not lower than 5 minutes (Vredenbregt & Rau, 1973). Mean walking speed was verified using a photoelectric timing system, with sensors positioned 0.95 m apart, at floor level, on both sides of the force plate. Pressure transducers were placed on default anatomic locations (calcaneous centre and first metatarsophalangeal joint), which helped to measure the gait cycle time of the leg which had no contact with the plate (Norkin & Levangie, 1992). All subjects used the same shoe type, in their size.

2.3.2 Measurement

All subjects walked along a 10 m walkway, as 10-12 m is the preferable interval to measure gait of young people, since it permits fast walkers to 'get into their stride' before any measurements are made (Whitle, 2007). Subjects were instructed to step on a force plate located in the middle of the walkway and to keep walking past the reference point without stopping. In each measurement, only the limb on which the GRF was measured had full contact with the plate and there was no extra load on it. Subjects walked for a minimum of 8 steps (James, et al., 2007; Oggero et al., 1998). According to the concept of gait optimisation, it is hypothesised that the neuromuscular locomotor system is best stabilised at the usual walking speed, that is to say, gait variability is also minimised at the usual walking speed (Masani, et al., 2002; Sekiya et al., 1997; Shiavi, et al., 1987a). Therefore, walking speed was freely chosen by each subject. Before the data acquisition session itself, subjects executed several trials to get used to the procedures.

EMGa data were collected by a one-channel unit at 1000 Hz. The signals were pre-amplified at the electrode site and then fed into a differential amplifier with an adjustable gain setting (12 - 500 Hz; CMRR: 95 dB at 60 Hz, input impedance of 100 MΩ and gain of 1000). Raw signals were digitised and stored on computer disks for subsequent analysis by the Acqknowledge software. After measurement, the EMG signal of MG during propulsion was processed and analised. Propulsion was defined as the time between the beginning of weight transfer from the calcaneous to the first metatarsophalangeal joint and the maximum peak of load of the first metatarsophalangeal joint. To guarantee valid results, we have previously taken measurements in each limb both with pressure (foot) switches and with the force plate. Comparing the signals obtained, we have concluded that by positioning pressure switches according to the indicated, we could use the defined time window to assess the EMG activity of the GM during propulsion. The EMG signal of MG during propulsion was filtered digitally with a zero-lag, second-order Butterworth filter with an effective band pass of 20–500 Hz and the root mean square (RMS) was calculated (Turker, 1993). The signal was also normalised according to maximal voluntary contraction to reduce subject variability and to convert the EMG amplitude to an estimate of muscle activation (Medved, 2001). Following a warm-up consisting of three submaximal isometric contractions, each subject was instructed to perform one series of three trials of maximal isometric plantar flexion force. Subjects were standing with the hip at 90º, the knee extended and the ankle in neutral position. They were asked to execute maximal isometric force for plantar flexion, under resistance, during 5 seconds, with one-minute rest between trials, being confirmed that there was no EMGa (Brown & Weir, 2001). The signals collected within the first and last seconds of each 5 seconds of isometric contraction were not used for analysis because of the possible occurrence of ankle movement at the initiation and completion of the test. Therefore, a 3-second window of EMG signal was used for analysis. This window of raw EMGa was processed using the RMS procedure to assess the electrical activity of the MG muscle.

GRF components (F_x, F_y and F_z) were filtered with a Butterworth filter and normalised according to weight (Masani, et al., 2002; Mullineaux, et al., 2006). The maximum value of the heel strike impulse peak in the F_z, F_y and F_x trace were used for analysis. To account for possible effects due to anthropometrics, gait speed was normalized to leg length (Hof, et al., 2002; Kim & Eng, 2004). To reduce the within-individual variability and increase statistical power, the calculated variables for the three trials for each subject were averaged (Mullineaux et al., 2001).

2.4 Statistics

Data analysis was performed using the Statistical Package Social Science (SPSS), version 13.0, from SPSS Inc. (USA). Shapiro-Wilk test results and Histogram analysis have shown that data were normally distributed; as a result, we have used parametric statistics. The Paired-Samples T Test was applied to assess possible significant differences between dominant and non-dominant limbs in terms of EMGa during propulsion and GRF during heel strike. The Pearson Correlation Coefficient Test was used to assess the correlation between MG EMGa and GRF and between EMGa/GRF and speed.

3. RESULTS

Figures 1-3 demonstrate the correlation between the EMGa of the non-dominant leg during propulsion and the GRF of the dominant leg during heel strike and between the EMGa of the dominant leg during propulsion and the GRF of the non-dominant leg during heel strike. According to the Pearson Correlation Coefficient Test, there was moderate correlation between the MG EMGa of the dominant leg during propulsion and F_z and F_y of the contra-lateral leg during heel strike ($r=0.797$, $p<0.0001$; $r=-0.807$, $p<0.0001$) and weak and moderate correlation between the MG EMGa of the non-dominant leg during propulsion and F_z and F_y, respectively, of the dominant leg during heel strike ($r=0.442$, p=0.018; r=-0.684, p<0.0001). These correlations indicate that the amount of variability in MG EMGa of the dominant leg explained by F_z and F_y of the contra-lateral leg is 63.5% and 65.12%, and that the amount of variability in MG EMGa of the non-dominant leg explained by F_z and F_y of the contra-lateral leg is 19.5% and 46.8%, respectively. Figure 4 shows how raw EMG signal and GRF profile vary for both limbs. No statistically significant correlations were observed between F_x of the dominant leg and EMGa of the contra-lateral leg (r=0.189, p=0.276) and between F_x of the non-dominant leg and EMGa of the contra-lateral leg (r=-0.184, p=0.291).

Although subjects walked at their comfortable speed and values of standard deviation between subjects were low, it was important to analyse the influence of speed variation on EMGa and GRF. The Pearson Correlation Coefficient Test showed a non-significant correlation between speed and EMGa of dominant (r=0.104, p=0.551) and non-dominant limbs (r=-0.187, p=0.282) and between speed and F_z, F_y and F_x of dominant (r=-0.102, p=0.558; r=-0.192, p=0.269; r=-0.284, p=0.099) and non-dominant limbs (r=0.055, p=0.792; r=-0.39, p=0.823; r=-0.017, p=0.923).

Comparing EMGa and GRF values obtained in dominant and non-dominant limbs (Table 1), there is not enough statistical evidence to conclude that there are significant differences in F_z and F_y at heel strike (p=0.18; p=0.358) and MG EMGa during propulsion (p=0.08). Differences were observed between F_x of dominant and non-dominant limbs at heel-strike (p<0.0001).

Table 1 – Mean and standard deviation values of EMGa during propulsion and GRF at heel strike in dominant and non-dominant members and speed.

Trial	Component	N	Mean	Standard deviation
1	Dominant EMG	35	0.3110	0.0689
	Non-dominant:			
	F_z		1.0240	0.10960
	F_y		0.0917	0.01677
	F_x		0.0691	0.02771
	Speed		0.3010	0.04930
2	Non-dominant EMG		0.3330	0.06090
	Dominant:			
	F_z		1.0120	0.10570
	F_y		0.0941	0.01421
	F_x		0.0811	0.02329
	Speed		0.4490	0.06060

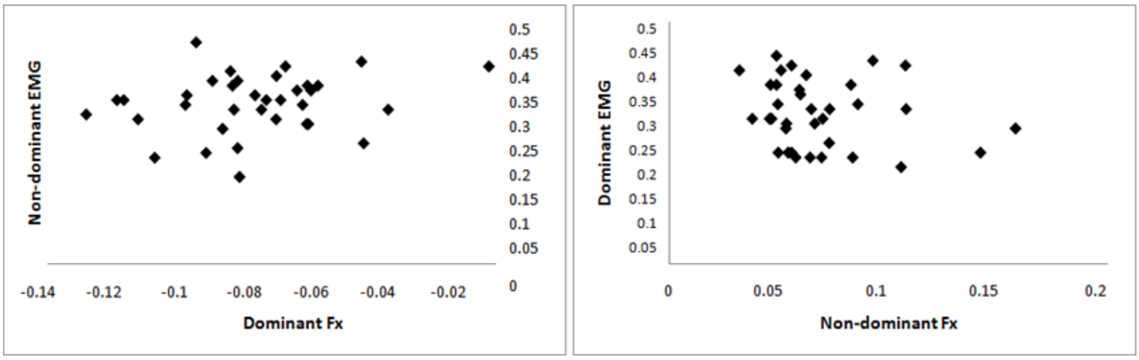

Figure 1 – Correlation between MG EMGa of the non-dominant limb during propulsion and F_x of the dominant limb during heel strike (right), and between MG EMGa of the dominant limb during propulsion and F_x of the non-dominant limb during heel strike (left).

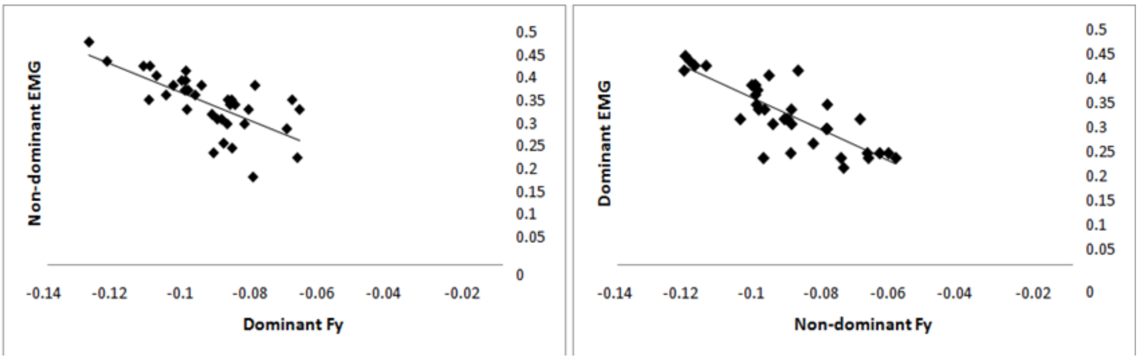

Figure 2 – Correlation between MG EMGa of the non-dominant limb during propulsion and F_y of the dominant limb during heel strike (right), and between MG EMGa of the dominant limb during propulsion and F_y of the non-dominant limb during heel strike (left).

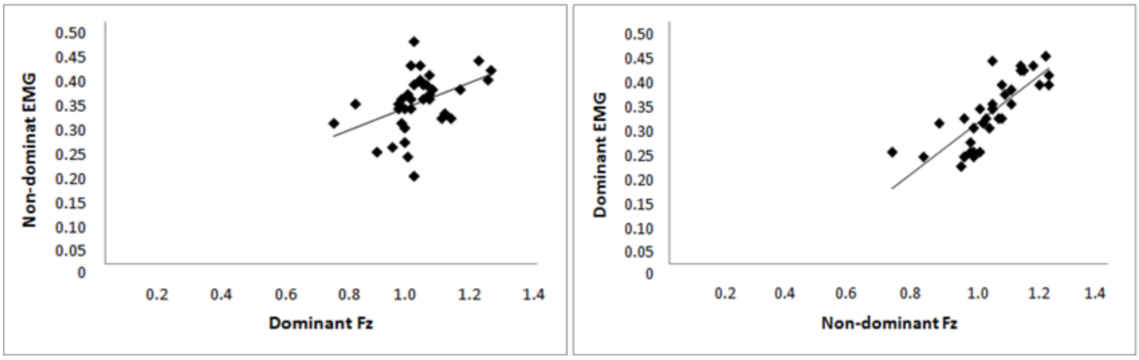

Figure 3 – Correlation between MG EMGa of the non-dominant limb during propulsion and F_z of the dominant limb during heel strike (right), and between MG EMGa of the dominant limb during propulsion and F_z of the non-dominant limb during heel strike (left).

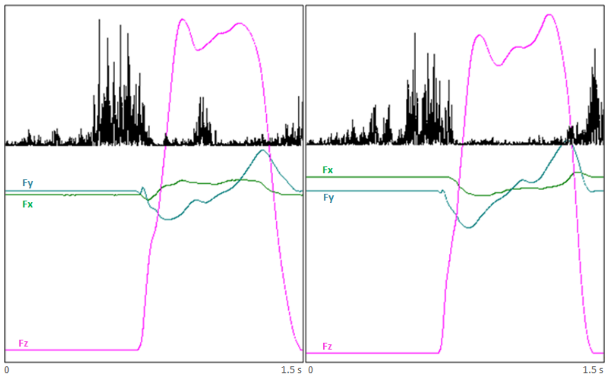

Figure 4 – Representation of one subject depicting how the raw EMGa of MG and the GRF profile vary for both limbs during double-support phase. At the left, one can see the absolute values of raw EMG signal of MG of the dominant leg (black) and the F_z (magenta), F_y (cyan) and F_x (green) of the non-dominant leg. At the right, the absolute values of raw EMG signal of MG of the non-dominant leg and the F_z, F_y and F_x of the dominant leg are shown.

4. Discussion and Conclusions

The importance of active work during propulsion (Doke & Kuo, 2007; Hill, 1953; Simon, et al., 1978) leads to the need of understanding the mechanisms involved in step-to-step transition, and more specifically to assess the influence of the contra-lateral leg heel strike on the degree of ankle plantar flexors' muscle activity.

Experiments in this study have shown a statistically significant correlation between the MG EMGa of the dominant leg during propulsion and F_z and F_y of the contra-lateral limb during heel strike and between the MG EMGa of the non-dominant leg and F_z and F_y of the contra-lateral limb at heel strike. According to the double-inverted pendulum model, the activity of the leading leg in the double support phase can be designated by heel strike, as the force directed along the leg executes negative work. On propulsion of the trailing leg, an equal amount of positive work is performed, arousing the need to restore energy loss in the following heel strike. Transition between steps reaches an optimum level when propulsion and heel strike have the same magnitude and a short duration (Kuo, et al., 2007). Looking at the step-to-step mechanism presented in (Kuo, et al., 2005, 2007), the results of this study suggest that F_z and F_y are associated to the amount of activity required by the MG of the contra-lateral limb, which is consistent to the role of plantar flexors during propulsion, as they have been considered important contributors to vertical and horizontal acceleration (Liu, et al., 2006; Neptune, et al., 2001; Winter, 1991; Zajac, et al., 2003). This finding corroborates the concept that the power activity of the trailing leg (propulsion) is related to that of the leading leg (stabilisation), and that the interaction between muscle powers during gait can reflect specific propulsion and control strategies that are related to each limb (Sadeghi et al., 2000).

In this study, ankle plantar flexor activation timing has not been analysed; however, it seems that the activity of medial gastrocnemius activity preceded the contra-lateral heel strike, which is not surprising since, when a muscle is activated, it takes time before the muscle force is fully developed. The time taken to reach maximum force depends on factors such as muscle fiber type, activation level and contraction dynamics, but for isometric contractions, it ranges between 23-73 ms (Burke et al., 1973; Burke et al., 1971; Gonyea et al., 1981). The preactivation period occurred before heel strike, and muscle activity within this period, is the result of feedforward control mechanisms. This is consistent with the evidence that the spinal co-ordination of bilateral leg muscle activation depends on facilitation by supraspinal centres. Indeed, cerebellar contribution via reticulo-spinal neurons has been suggested in humans (Bonnet et al., 1976) and recent evidence was presented for a cortical (supplementary motor area) control of interlimb co-ordination (Debaere et al., 2001). Considering the information pointed above, it would be important in future studies to analyse plantar ankle flexors timing activity during double support.

It is becoming more and more accepted that, in addition to neural mechanisms, the mechanical properties of the body play a primary role in the dynamics and intrinsic frequencies with the complex nonlinear properties, to which the frequencies, phases and shapes of motoneuron signals must be adapted for efficient locomotion and motor control (Rybak, 2006). The results of this study demonstrate that there is a relation between the mechanics of the leading leg and the muscle activity of the trailing leg during step-to-step transition. The importance of ankle muscle activity in step-to-step transition is expressed in studies dedicated to this mechanism in transtibial amputed subjects (Houdijk et al., 2009) and subjects with total ankle arthroplasty (Doets, et al., 2009). These studies indicate that ankle impairment leads to a decrease of positive work by the trailing leg and a consequent increase of negative work by the leading leg, which partially explains the increased metabolic cost of walking. The results of our study demonstrate a higher correlation between MG EMGa and F_y, which corroborates its major importance in forward propulsion (Gottschall & Kram, 2003). On the other hand, no significant correlation was observed between F_x at heel strike and MG EMGa during propulsion, which can result not only from the fact that MG major role is related to trunk support and forward displacement (Liu, et al., 2006; Neptune, et al., 2001) but also from the fact that F_x is the highest variable component (Winter, 1991). This higher variability can explain the differences observed between dominant and non-dominant limbs at heel strike.

As to the results obtained, there are two important questions that need discussion. First, different values of Pearson correlation have been noted between the EMGa of dominant and non-dominant limbs and the GRF of contra-lateral limbs (the first have presented a moderate correlation and the second only a weak correlation). Differences between dominant and non-dominant limbs have been reported frequently, as lower limbs are not used equally during walking (DuChatinier & Rozendal, 1970). This asymmetry has been interpreted based on the support and mobility associated to each limb (Hirasawa, 1979, 1981; Vanden-Abeele, 1980), as one leg contributes more to propulsion while the contra-lateral one is mainly responsible for support and body weight transfer during walking (dominant and non-dominant limbs, respectively) (Dargent-Pare et al., 1992; Gabbard, 1989; Peters, 1988). Therefore, it can be hypothesised that the higher correlation between the EMGa of the dominant limb and F_z and F_y of the contra-lateral limb results from the fact that the dominant limb contributes more to propulsion, and so it is more adapted to this function. On the other hand, evidence suggests that the dominant leg is stronger in plantar flexion (Damholt & Termansen, 1978) which allows accepting that during the double support phase, it is more related to F_z and F_y of the contra-lateral leg than the non-dominant limb. The second question is related to the growing evidence showing the compartmentalisation of the human gastrocnemius (McLean & Goudy, 2004; Staudenmann et al., 2009; Vieira et al., 2010; Wolf & Kim, 1997). It has been demonstrated that portions of the same gastrocnemius muscle are activated differently, depending on the direction of the ankle force (Staudenmann, et al., 2009), and that surface EMGs recorded from the pinnated MG muscles are extremely selective (Vieira, et al., 2010). Taking this information into account, it would be important, in future studies, to analyse the different activation patterns from distinct parts of the triceps surae muscle, as the possibility of having specific, localised MG regions involved in limb propulsion could be related to the finding that F_z and F_y only explain part of the MG EMGa.

Several studies agree that changes in walking speed are associated with increases in the intensity of muscle activation (Crowe, et al., 1993, 1995; den Otter, et al., 2004; Ivanenko, et al., 2006). The results of this study show that speed differences obtained between subjects were not related to MG EMGa and GRF. These findings can be explained by the fact that subjects walked at their own comfortable speed and mean values obtained were according to reference values (Bohannon, 1997); in addition, standard deviation values were low, which is related to the high homogeneity of the sample. As to the influence of speed on GRF values, the results of this study are according to the ones obtained in (Keller et al., 1996), where the GRF increased linearly with gait speed only up to about 60% of the subjects' maximum speed. It is important to note that this study only addressed the correlation of subject walking speed on MG EMGa and GRF to exclude a possible effect of speed, as subjects were asked to walk at a comfortable speed. However, as changes in walking speed are associated with increases in the intensity of muscle activation and GRF magnitude, it would be important, in future studies, to analyse the influence of speed on the relation between MG EMGa of the trailing leg and GRF of the leading leg.

Another aspect that is important to note is related to the repeatibility of GRF peak measurements and limitations of the instruments. As stated in the instruments section, we have taken into account several considerations as to force platform mounting to avoid measurement errors. In addition, the coefficient of variation of GRF peak values obtained in each subject was almost always below 12.5%. Moreover, like in the present study, several other researchers used the GRF first peak value not only in healthy subjects (Cook et al., 1997; Masani, et al., 2002; Simpson & Jiang, 1999) but also in subjects with pathology (Chockalingam et al., 2004; Levinger & Gilleard, 2007; McCrory et al., 2001; Winiarski & Rutkowska-Kucharska, 2009) and even as a measure to control the influence of an exercise program (Nyland et al., 2011). However, considering limitations in terms of repeteability of GRF peak measurements, it would be important in future studies to analyse the relation between muscle activity of one limb and the slope of the transient of GRF of the contra-lateral limb during double support.

Considering that the EMGa of the trailing leg was correlated with the magnitude of F_z and F_y of the leading leg, it would be important, in future studies, to assess how much of the negative work produced during heel strike might be compensated by this muscle.

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PART B – *ARTICLE IV*

Interlimb relation during the double support phase of gait: an electromyographic, mechanical and energy based analysis

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ABSTRACT

The purpose of this study is to analyse the interlimb relation and the influence of mechanical energy on metabolic energy expenditure during gait. In total, 22 subjects were monitored as to electromyographic activity (EMG), ground reaction forces (GRF) and VO_2 consumption (metabolic power) during gait. The results demonstrate a moderate negative correlation between the activity of tibialis anterior (TA), biceps femoris (BF) and vastus medialis (VM) of the trailing limb (TRAIL) during mid-stance to double support transition (MS-DS) and that of the leading limb (LEAD) during DS for the same muscles, and between these and gastrocnemius medialis (GM) and soleus (SOL) of the TRAIL during DS. TRAIL SOL during MS-DS was positively correlated to LEAD TA, VM and BF during DS. Also, the TRAIL centre of mass (CoM) mechanical work was strongly influenced by the LEAD's, although only the mechanical power related to forward progression of both limbs was correlated to metabolic power. These findings demonstrate a consistent interlimb relation in terms of EMG and CoM mechanical work, being the relations occurred in the plane of forward progression the more important to gait energy expenditure.

Key words: Metabolic energy, mechanical work, electromyography, gait, double support

1. INTRODUCTION

Human gait is influenced by a multifactorial interaction that results from neural and mechanical organisation, including musculoskeletal dynamics, a central pattern generator and peripheral and supraspinal inputs (Rossignol, et al., 2006). In spite of the complexity involved, all control mechanisms must be considered and discussed based on the need of controlling the body center of mass (CoM) over the base of support (Dietz, 1996).

Neuronal mechanisms, mediated at spinal level to achieve task-directed coupling of bilateral leg muscle activation, ensure the complex control of CoM movement (Dietz, 1996). In fact, experiments on interlimb coordination of leg muscle activation have confirmed that unilateral leg displacement during gait evokes a bilateral response pattern, with a similar onset in both sides (Corna, et al., 1996; Dietz, 1992) but only when both limbs are performing a supportive role (Dietz & Berger, 1984; Marchand-Pauvert, et al., 2005). This is consistent with the evidence that a large majority of midlumbar interneurones recipient from group II input are influenced by afferent fibres from both ipsilateral and contra-lateral sides (Bajwa, et al., 1992) and by vestibulo- and reticulo-spinal pathways (Davies & Edgley, 1994), and with the importance given to medium latency response from group II to feedback in the stance phase of gait (Grey, et al., 2001).

The transition from one stance limb inverted pendulum to the next appears to be a major determinant of the mechanical work of walking (Donelan, et al., 2002b; Kuo, et al., 2007) and occurs mainly during double support (DS), as the two leg forces need to redirect the CoM velocity from a downward and forward direction to an upward and forward direction. It has been demonstrated that a low percentage of energy recovery in the DS (Geyer, et al., 2006) is related to the interruption of the energy-conserving motion of single support by an inelastic collision of the swing leg with the ground, leading to changes in velocities of the legs and the CoM (Kuo, et al., 2007). This energy loss can be reduced by 75% through the application of a propulsion impulse in the trailing limb (TRAIL) immediately before collision of the leading limb (LEAD) (Kuo, 2002; Kuo, et al., 2007).

Simulations suggest that ankle plantar flexors and uni- and bi-articular hip extensors dominate the work output over the gait cycle (Neptune, Kautz, et al., 2004). These muscles, being active at late stance and at the beginning of stance, are therefore restoring energy to the body near DS (Zajac, et al., 2003). A previous study demonstrated that the ground reaction force magnitude of the LEAD is associated with the electromyographic activity of the contra-lateral medial gastrocnemius during DS (Sousa, Santos, et al., 2012); however, to the best of our knowledge, no prior study addressed the interdependence of the TRAIL and LEAD during DS in terms of muscle activity and CoM mechanical work. The understanding of this interlimb relation during walking is of interest to researchers involved in human movement science, but also to clinicians seeking to improve the walking ability of patients, particularity those presenting an asymmetric gait pattern, like post-stroke subjects.

The purpose of this study is to analyse the degree of interdependency of muscle activity and CoM mechanical work between TRAIL and LEAD during DS of walking and between mechanical energy of each limb and gait metabolic energy expenditure.

2. METHODS

2.1 Subjects

Twenty two adult subjects (10 males and 12 females) were recruited to participate in this study (age = 49.24±7.69 years, height = 1.66±0.09 m, body weight = 67.4±8.76 kg; mean±SD). The study excluded possible candidates presenting pain or with history of osteoarticular or musculotendon injury of the lower limb in the last 6 months, background or signs of neurological dysfunction or medication which could affect motor performance and history of lower limb surgery and lower limb anatomical deformities were excluded.

The study was approved by the local ethics committee and implemented according to the Declaration of Helsinki. All participants gave their written informed consent.

2.2 Instrumentation

The metabolic energy expenditure was measured by analysing inspired and expired air (K4b2 from COSMED, Italy). The values of vertical (F_z), anteroposterior (F_y) and mediolateral (F_x) components of ground reaction forces (GRF) were obtained from two force plates at a sampling rate of 1000Hz (models FP4060-10 and FP4060-08 from Bertec Corporation, USA, connected to a Bertec AM 6300 amplifier and to a Biopac 16-bit analogical-digital converter, from BIOPAC Systems, Inc. USA). The bilateral electromyographic activity (EMG) of Medial Gastrocnemius (MG), Soleus (SOL), Tibialis Anterior (TA), Rectus Femoris (RF), Vastus Medialis (VM) and Biceps Femoris (BF) was monitored using surface EMG sensors (emgPLUX model from Plux Ltda, Portugal). The signals collected with a sampling frequency of 1000 Hz were pre-amplified at the electrode and then fed into a differential amplifier with an adjustable gain setting (25 - 500 Hz; common-mode rejection ratio (CMRR): 110 dB at 50 Hz, input impedance of 100 MΩ and gain of 1000). For the analog to digital signal conversion and bluetooth transmission to the computer, a wireless signal acquisition system (bioPLUX research, Plux Ltda) was used. Self-adhesive silver chloride EMG electrodes (Dahlausen 505) were used in a bipolar configuration and with a distance of 20 mm between detection surfaces (centre to centre). Skin impedance was measured with an Electrode Impedance Checker (Noraxon USA, Inc.). The EMG and force platform signals were digitised and stored for subsequent analysis in the Acqknowledge software (Biopac Systems, Inc., U.S.A). Gait timing was measured using a photovoltaic system (Brower Timing, IRD-T175, USA). All subjects used the same shoe type, in their adequate size.

2.3 Procedures

2.3.1 Skin preparation and electrode placement

The skin surface of the selected muscles mid-belly and of the patella was prepared (shaved, dead skin cells and non-conductor elements were removed with alcohol and with an abrasive pad) to reduce the electrical resistance to less than 5000Ω. EMG electrodes were placed according to anatomical references (Table 1).

Table 1: Anatomical references to electrode placement. Electrode locations were confirmed by palpation of the muscular belly with the subject in the test position, being the electrodes placed on the most prominent area.

Muscle	Electrode placement
TA	1/3 on the line between the tip of the tibia and the tip of the medial malleolus
GM	Most prominent bulge of the muscle
SOL	2 cm distal to the lower border of the medial gastrocnemius muscle belly and 2 cm medial to the posterior midline of the leg
RF	1/2 on the line from the anterior spina iliaca to the superior border of the patella
VM	4 cm above the patella upper border and 3 cm measured medially and oriented 55º to a reference line drawn between the right antero-superior iliac spine and the patella centre
BF	1/2 on the line from the ischial tuberosity and the lateral epicondyle of the tibia
Ground electrode	Patella centre

2.3.2 Data acquisition

a) Kinetic and electromyographic data

EMG and GRF data were acquired while participants were walking using standard footwear over a 10 m walkway that included two separate force plates mounted in series near the midpoint of the walkway. Before data acquisition, sufficient time was given so that participants became familiar with the experimental settings. They were allowed to walk over the walkway without explicit instructions, while we observed the starting point on the walkway where they placed one foot on the first force plate and the other on the second force plate according to their natural cadence. Considering that at the usual gait speed gait variability is minimised (Masani, et al., 2002; Sekiya, et al., 1997; Sousa & Tavares, 2012a), the walking trials were carried out at a self-selected speed. The average values of 3 successful trials were used for analysis. A trial was considered successful when one foot had full contact with the first plate (TRAIL) and the contra-lateral limb had full contact with the second platform (LEAD).

b) Metabolic energy expenditure data

Subsequently, metabolic energy expenditure by each participant was measured while walking on a treadmill at the speed adopted during the walkway trials, since no differences in energy expenditure have been reported between these two conditions (Ralston, 1960). Before initiating treadmill walking, the resting metabolism was measured during 3 minutes of quiet standing. A 3-5 minutes interval was set for subjects to reach a steady state during walking (Gottschall & Kram, 2003). After this period, participants walked for 3 more minutes while oxygen uptake was measured (Doets, et al., 2009). Then, the standing values were subtracted from walking values. Halfway during each treadmill trial, the step frequency was registered and it was verified that it was similar to the one obtained in the walkway test.

2.3.3 Data processing

a) Electromyographic activity

The EMG signals of the TRAIL and LEAD muscles during the transition between mid-stance and double support (MS-DS) and DS (Figure 1) were filtered using a zero-lag, second-order Butterworth filter with an effective band-pass of 20-450 Hz and the root mean square (RMS) was calculated. The EMG signals for each subject were normalised to the mean signal for each muscle over the entire gait cycle (Yang & Winter, 1984).

b) Kinetic parameters

GRF data were low-pass filtered using a cutoff frequency of 8 Hz, with a fourth-order Butterworth filter by using a zero-lag and the CoM mechanical work was calculated for DS (Figure 1) according to the individual limbs method proposed by (Donelan, et al., 2002b). In brief, the CoM accelerations in the three orthogonal directions were obtained from the sum of the GRF acting under each limb using the second law of Newton. Then, the CoM velocity was found by integrating acceleration over time. Integration constants were chosen under the following assumptions: the average vertical CoM velocity over a complete step is zero, average fore-aft velocity is equal to the average walking speed and medio-lateral CoM velocity at the beginning and end of the step is equal in magnitude but opposite in sign. A step was defined as the time between the beginning of one foot contact with the ground and the beginning of the opposite foot contact with the ground (Donelan, et al., 2002b), which includes the first double support and single leg stance. The external mechanical work generated on the CoM by the TRAIL and LEAD individually was determined using the time integral of the dot product of the GRF of each limb and the velocity of the CoM and was normalized to the body mass of the subject (J/Kg). With this calculation of mechanical work, the net mechanical work was calculated for DS in both TRAIL and LEAD. To compare average mechanical work with the metabolic power, the mechanical work (J/Kg) was divided by the stride time (s) (defined as the time between initial ground contact in the first force plate and a sudden large displacement of the centre of pressure on the second force plate) to obtain average mechanical power (W/Kg). For each participant, the data of 3 successful trials for each leg were averaged after analysis. Only CoM external work was calculated as it estimates satisfactorily the total CoM mechanical work performed, as during DS the angular displacements of the limbs relative to the CoM are relatively small, indicating that there is little internal work (Donelan, et al., 2002b). EMG and mechanical CoM work and power data analysis was performed using Matlab software (MathWorks, USA).

c) Metabolic energy expenditure measurement

The metabolic energy consumption $(\dot{E}_{met})$ was calculated from the VO_2 (ml/s) and respiratory exchange ratio (RER) values (Doets, et al., 2009):

$$\dot{E}_{met} = 4.94 \times RER + 16.04 \times VO_2.$$

To quantify the metabolic power (W/kg), the metabolic energy consumption $(\dot{E}_{met})$ was divided by the body mass of the subject (kg).

Only the mechanical power and metabolic power were calculated as these variables have been demonstrated to be correlated (Doets, et al., 2009; Houdijk, et al., 2009), and because the mechanical power has been described as the major time-varying variable that can be tracked from mechanical energy based analysis (Winter & Eng, 1995).

2.4 Statistics

The acquired data were analysed using the software Statistic Package Social Science (SPSS) from IBM Company (USA). Correlation between TRAIL and LEAD EMG activity was analysed using the Spearman’s correlation coefficient and the correlation between CoM mechanical work of TRAIL and LEAD and between the metabolic and mechanical power was analysed using the Pearson's correlation coefficient. The Paired Samples T-test was used to compare the mechanical work and mechanical power between limbs. Statistical significance was set at $p<0.05$.

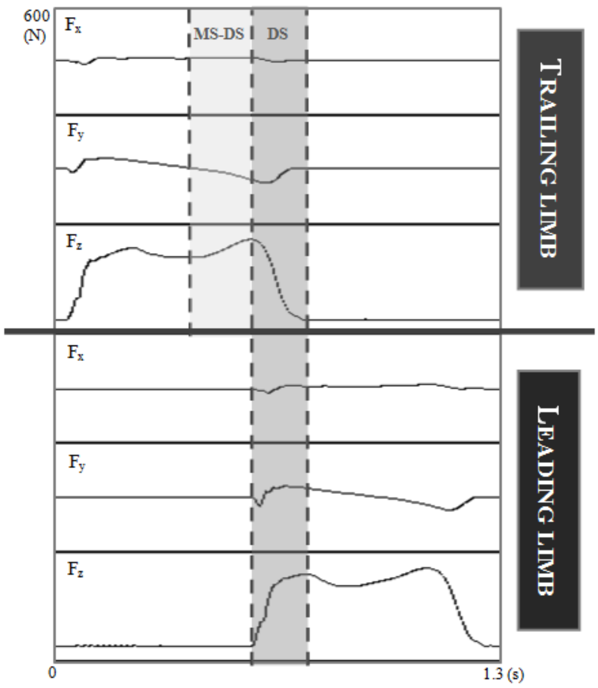

Figure 1: Representation of the stance subphases defined according to the GRF curve. MS-DS of the TRAIL was defined as the time between the instant when the F_y of the TRAIL assumes the value zero till the instant when the F_z of the LEAD assumes a value equal to or higher than 7% of body weight; in this subphase, the EMG of TRAIL muscles was analysed. DS was defined as the time between the instant when the F_z of the LEAD assumes a value equal to or higher than 7% of body weight till the instant when the F_z of the TRAIL assumes a value equal to or lower than 7% of body weight.

3. RESULTS

3.1 EMG during MS-DS and DS and CoM mechanical work during DS: interlimb relation

Values in Table 2 indicate that the higher the activity of TA, VM and BF during TRAIL MS-DS the lower the activity of the same muscles during LEAD DS. Also, TRAIL SOL activity during MS-DS was positively correlated to activity of TA, BF, VM and RF of the LEAD. The same muscles of the LEAD were the most correlated to the activity of the TRAIL during DS. Specifically, the higher the activity of TA, RF and VM of the LEAD the lower the activity of SOL and GM of the TRAIL during DS, and the higher the LEAD BF the lower the TRAIL SOL.

In line with the results obtained for the EMG, a strong dependency between TRAIL and LEAD during DS was also observed in terms of CoM mechanical work (Table 3). Findings suggest that the higher the absolute value of the negative LEAD CoM mechanical work, the higher the absolute value of the positive TRAIL CoM mechanical work. 7TRAIL and LEAD CoM mechanical work was compared (Table 4) with the differences occuring only in the anteroposterior component, being higher in the TRAIL. Despite the interlimb relation observed as to EMG and CoM mechanical work, no statistical significant correlations were observed between EMG and CoM mechanical work.

Table 2: Correlation coefficient values and p-values obtained between muscle activity of LEAD during DS and of TRAIL during MS-DS and DS.

			LEAD during *DS*					
			GM	**SOL**	**TA**	**BF**	**RF**	**VM**
TRAIL	**GM**	*MS-DS*	NS	NS	NS	NS	NS	NS
		DS			r=-0.521 p=0.009		r=-0.452 p=0.027	r=-0.419 p=0.041
	SOL	*MS-DS*			r=0.603 p=0.001	r=0.511 p=0.011	r=0.507 p=0.011	r=0.455 p=0.026
		DS			r=-0.670 p<0.001	r=-0.554 p=0.005	r=-0.652 p=0.001	r=-0.668 p<0.0001
	TA	*MS-DS*			r=-0.577 p=0.003	r=-0.650 p=0.001	NS	r=-0.676 p<0.0001
		DS			Ns	Ns		ns
	BF	*MS-DS*			r=-0.425 p=0.038	r=-0.655 p=0.001		r=-0.639 p=0.001
		DS			NS	NS		NS
	RF	*MS-DS*						
		DS						
	VM	*MS-DS*			r=-0.423 p=0.039	r=-0.517 p=0.010		r=-0.581 p=0.003
		DS			NS	NS		NS

3.2 Mechanical power during DS and metabolic power

The values obtained as to metabolic and mechanical power are shown in Table 4. Metabolic power was strongly related to positive mechanical power by the TRAIL ($r=0.731$, $p<0.001$) and negative mechanical power by the LEAD ($r=-0.723$, $p<0.001$) (Figure 2). As with CoM mechanical work, higher anteroposterior mechanical power was observed in the TRAIL in relation to the LEAD during DS, while no differences were observed in the other components (Table 4).

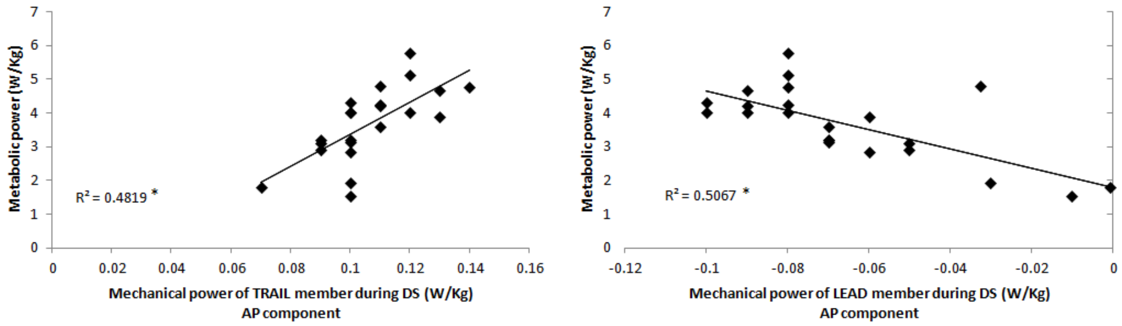

Figure 2: Correlations found between mean metabolic power and AP mechanical power during DS (*significant correlation ($p<0.05$)).

Table 3: Correlation coefficient values and p-values obtained between CoM mechanical work of LEAD during DS and of TRAIL during MS-DS and DS.

		LEAD CoM Mechanical work			
		Mediolateral	*Anteroposterior*	*Vertical*	*Total*
TRAIL CoM Mechanical work	*Mediolateral*	NS	---	---	---
	Anteroposterior	---	r=-0.657 p<0.001	---	---
	Vertical	---	---	r=-0.890 p<0.001	---
	Total	---	---	---	r=-0.890 p<0.001

Table 4: Mean and standard deviation (SD) values of CoM mechanical work and power during DS and metabolic power of gait (*$p<0.05$).

Variable	Component	Limb	Mean ± SD	p-value
CoM Mechanical work ($J\ Kg^{-1}$)	*Mediolateral*	TRAIL LEAD	0.003 ± 0.001 -0.002 ± 0.0003	0.076
	Anteroposterior	TRAIL LEAD	0.12 ± 0.005 -0.08 ± 0.007	<0.001*
	Vertical	TRAIL LEAD	0.67 ± 0.057 -0.70 ± 0.063	0.265
	Total	TRAIL LEAD	0.70 ± 0.054 -0.68 ± 0.041	0.954
Mechanical power ($W\ Kg^{-1}$)	*Mediolateral*	TRAIL LEAD	0.003 ± 0.001 -0.002 ± 0.001	0.135
	Anteroposterior	TRAIL LEAD	0.11 ± 0.005 -0.07 ± 0.006	<0.001*
	Vertical	TRAIL LEAD	0.60 ± 0.04 -0.65 ± 0.06	0.057
	Total	TRAIL LEAD	0.66 ± 0.06 -0.62 ± 0.04	<0.001*
Metabolic power ($W\ Kg^{-1}$)	----	----	3.63 ± 0.25	----

4. DISCUSSION

This study aimed to assess the interlimb relation in terms of muscle activity and CoM mechanical work. Another purpose was to evaluate the influence of mechanical energy of each limb over metabolic cost of walking.

The results obtained as to the electromyographic activity reveal a consistent interlimb relation. It was interesting to note that the LEAD TA, VM and BF during DS depended, based on an inverse relation, on the activity of the same muscles of the TRAIL during MS-DS. The role of TA, hamstrings, gluteus maximus and quadriceps muscles of the LEAD has been stressed for the initial phase of stance (Zajac, et al., 2003). From these, TA, hamstrings and quadriceps (specifically the vasti muscles) seem to be the most related to impact reduction during heel strike, having a non-determinant role in forward propulsion during MS-DS. The relation mentioned is therefore consistent to the evidence that the best proprioceptive information may come from un-modulated muscles (Di Giulio, et al., 2009). Also, higher levels of TRAIL SOL activity during MS-DS were related to higher activity of the main responsible for LEAD impact reduction. Considering the agonist role of plantar flexors in forward propulsion (Gottschall & Kram, 2003; Neptune, et al., 2001; Neptune, Kautz, et al., 2004), these finding suggest that the higher the TRAIL forward propulsion during MS-DS the lower the LEAD impact. However, considering that EMG measurements alone could not provide information on the absolute contributions to the mechanical output of each muscle, or the relative contributions within a given condition, we suggest the combination of EMG with other analysis techniques, such as, computational modeling and simulation, to assess clearly the relation between TRAIL individual muscle function during MS-DS and LEAD impact during heel strike.

The results of this study also demonstrate that the lower the muscle activity related to impact reduction during heel strike (VM and TA) the higher the muscle activity related to forward propulsion during push-off (GM and SOL), corroborating models predicting that the TRAIL compensates for the energy loss provoked by the LEAD during heel strike through the action of plantar flexors (Donelan, et al., 2002b; Kuo, 2002; Kuo, et al., 2007). This inverted relationship is consistent with the correlation observed in CoM mechanical work between limbs, as the higher the negative CoM mechanical work performed by the LEAD the higher the positive CoM mechanical work performed by the TRAIL. Assuming that gait kinematics remain similar between subjects at the velocity adopted in this study, then increases or decreases of muscle activity would be related to muscle mechanical output (McGowan et al., 2009). Considering the double inverted pendulum model, the activity of TA, VM and BF muscles would be associated to a reduction of the LEAD negative CoM mechanical work, and that of GM and SOL to an increase of the TRAIL positive CoM mechanical work. However, our results failed in demonstrating an association between the activity of these muscles and CoM mechanical work. This can be due to the fact that the relationship between muscle activity and mechanical output depends on a number of nonlinear intrinsic properties, i.e., force-length-velocity relationships that make the relationship difficult to predict (McGowan, et al., 2009). Given this limitation, it would be important to analyse the relation of joint moment power between limbs during DS and between the CoM mechanical work of each limb. Also, the kind of normalisation procedure used could be related to these results as, in spite of being useful in reducing inter-subject variability, it does not reflect the percentage of the maximum neural drive (Sousa & Tavares, 2012b). This may explain the correlation found in a previous study between GM activity of the TRAIL and the magnitude of GRF of the TRAIL (Sousa, Santos, et al., 2012). As such, it would be important in future studies to analyse this relation using the maximum voluntary normalisation method.

The importance of the relation found between muscle activity of TRAIL and LEAD was reinforced by the strong influence of the anteroposterior component of negative (LEAD) and positive (TRAIL) mechanical power over the metabolic power of walking. Indeed this finding is in line with the results presented by Gottscall and Kram, 2003, as to the influence of horizontal propulsive forces in metabolic cost of walking, and with results obtained in subjects with ankle impairment, as it was observed that both decreased TRAIL positive work (Houdijk, et al., 2009) and increased LEAD negative work (Doets, et al., 2009) were related to increased metabolic cost of walking.

5. CONCLUSION

The results of this study demonstrate that while muscle activity of the TRAIL during MS-DS determined muscle activity of the LEAD during DS, LEAD activity determined the activity of the TRAIL during DS. Also, the TRAIL CoM mechanical work was influenced by the LEAD's, but only the mechanical power related to forward progression of both limbs was related to metabolic power.

The results obtained highlight the importance of plantar flexors performance in gait economy, as they are the main responsible for forward propulsion, compensating the energy loss during the LEAD heel strike, but they also interfere with the level of activity of the LEAD muscles responsible for impact reduction during heel strike. Considering that low plantarflexor strength and power are common features in stroke subjects, future work will focus on quantifying muscle function and interlimb relation during DS of post-stroke gait.

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PART B – *ARTICLE V*

Interlimb coordination during the stance phase of gait in stroke patients

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Submitted to an International Journal

ABSTRACT

Background and Purpose: Interlimb coordination assumes an important role in gait energy consumption. The purpose of this study was to analyse the relationship between the contra-lateral (paretic) and ipsilateral (non-paretic) limbs of the affected hemisphere in patients with stroke during step-to-step transition in walking.

Methods: The electromyographic activity (EMG) of the lower limb muscles and ground reaction forces of 16 patients with stroke and 22 healthy controls were monitored during gait. The soleus (SOL), gastrocnemius medialis (GM), tibialis anterior (TA), biceps femoris (BF), rectus femoris (RF) and vastus medialis (VM) muscles were recorded.

Findings: The propulsive impulse of the paretic trailing limb was negatively correlated to the braking impulse of the leading limb during double-support ($r=-0.639$, $p=0.010$). A moderate functional relationship was observed between thigh muscles ($r=-0.529$, $p=0.035$), and a strong and moderate dysfunctional relationship was found between the plantar flexors of the non-paretic limb and the vastus medialis of the paretic limb (SOL-VM, $r=-0.800$, $p<0.001$; GM-VM, $r=-0.655$, $p=0.002$). Also, a functional moderate negative correlation was observed between the SOL muscle of the non-paretic limb during transition between midstance and double-support (MS-DS) and the SOL ($r=-0.506$, $p=0.046$) of the paretic limb during heel strike and between the RF muscle of the non-paretic limb during MS-DS and the VM ($r=-0.518$, $p=0.040$) muscle of the paretic limb during heel strike. The impulse contribution of the paretic trailing limb was lower than that of the non-paretic trailing limb in patients with stroke ($p=0.02$) and in healthy subjects ($p=0.008$).

Discussion and Conclusions: The findings obtained seem to suggest that the lower performance of the paretic limb in forward propulsion during gait is not only related to contra-lateral supraspinal damage, but also to a dysfunctional influence of the non-paretic limb.

Keywords: step-to-step transition; electromyographic activity; propulsive impulse; patients with stroke.

1. INTRODUCTION

Gait disorders affect a large proportion of patients with stroke (Duncan, et al., 2005; Wevers, et al., 2009), limiting their ability to ambulate in the community (Keenan et al., 1984). The characteristics of post-stroke walking vary according to stroke severity, location of the infarct, time since stroke, type of rehabilitation received and other individual differences (Olney & Richards, 1996; Richards & Olney, 1996). However, higher mechanical energy cost per stride (Chen et al., 2005a; Detrembleur, et al., 2003) and metabolic energy expenditure (Corcoran et al., 1970) are typically found in patients with stroke than in healthy subjects.

The role of interlimb coordination during the double-support phase (DS) in unimpaired gait energy consumption (Doets, et al., 2009; Donelan, et al., 2002a) is of great importance for biomechanical models because the transition from one stance limb inverted pendulum to the next appears to be a major determinant of the mechanical work involved in walking (Donelan, et al., 2002a; Kuo, et al., 2007). An optimal mechanical relationship between human limbs has been described as the trailing limb (TRAIL) compensates the energy loss provoked by the leading limb (LEAD) during heel strike (Donelan, et al., 2002a; Kuo, et al., 2007), through the action of plantar flexors (Kuo, 2002; Kuo, et al., 2005) in order to maintain the velocity of the body's centre of mass. Recent studies have demonstrated that in healthy subjects unilateral plantar flexors' activity depends on muscle activity (Sousa, Silva, et al., 2012b) and ground reaction force magnitude (Sousa, Santos, et al., 2012) of the contra-lateral limb during DS. This interlimb relationship observed during step-to-step transition in unimpaired walking (Sousa, Santos, et al., 2012; Sousa, Silva, et al., 2012b), as well as in standing-related tasks (Corna, et al., 1996; Dietz, 1992), can be justified by the bilateral influence of group II fibres on spinal interneurons (Bajwa, et al., 1992) and by the influence of vestibulo- and reticulo-spinal pathways on group II fibres (Davies & Edgley, 1994). Taking into account that the importance of these afferences has been highlighted during the stance phase of gait (Grey, et al., 2001; Sinkjær, et al., 2000) and that the same afferences are disrupted after stroke (Marque, et al., 2001; Maupas, et al., 2004; Nardone & Schieppati, 2005), one might expect altered modulation of interlimb coordination in stroke patients; particularly, those with subcortical injuries in the middle cerebral artery territory, such as in the internal capsule (Jankowska, et al., 2003; Matsuyama, et al., 2004).

Patients with stroke present low kinetic energy (Chen & Patten, 2008; Chen, et al., 2005b) and an inadequate propulsion of the contra-lateral limb to the affected hemisphere (paretic limb) during pre-swing (Jonkers et al., 2009; Nadeau, et al., 1999; Peterson et al., 2010) as a result of low plantar flexor strength and power (Nadeau, et al., 1999; Olney & Richards, 1996). The major metabolic cost has been associated with the mechanical work done by the ipsilateral limb to the affected hemisphere (non-paretic limb) to lift the centre of mass (Stoquart et al., 2012). However, although the non-paretic limb has often described as a compensatory limb that adapts to changes in the paretic limb (Gaviria et al., 1996; Lamontagne, et al., 2002), there is the possibility of a dysfunction of the neuronal system over the non-paretic limb (Lamontagne, et al., 2002; Shiavi, et al., 1987b; Silva, Sousa, Pinheiro, Ferraz, et al., 2012; Silva, Sousa, Pinheiro, Tavares, et al., 2012; Silva, Sousa, Tavares, et al., 2012). The non-paretic limb function has been shown to have an impact on the paretic limb function during cycling. Specifically, the paretic limb had a lower performance when the task was executed with both limbs (Kautz & Patten, 2005). These findings suggest that not only the paretic limb leads to changes in performance of the non-paretic limb but also that the non-paretic limb may lead to performance changes in the paretic limb. This possibility is of significant relevance from a clinical and rehabilitation point of view, considering that rehabilitation strategies are focused only on the paretic limb.

The main purpose of this study was to analyse the relationship between non-paretic and paretic limbs during gait step-to-step transition in terms of individual muscle activity and global kinetic values in patients with stroke. Based on the findings obtained with neurophysiologically healthy subjects (Sousa, Silva, et al., 2012b) and the changes observed in paretic (Jonkers, et al., 2009; Lin, et al., 2006; Nadeau, et al., 1999; Peterson, et al., 2010) and non-paretic (Silva, Sousa, Tavares, et al., 2012) limbs during gait of patients with stroke, a poor interlimb coordination was hypothesised for these latter subjects. In general, the non-paretic limb would be expected to have a greater influence on the paretic limb rather than the opposite and that the non-paretic limb would exert a dysfunctional influence on the paretic limb.

To the best of our knowledge, no previous study has evaluated the interlimb relationships during gait in patients with stroke. Whereas correlation analysis has revealed that some EMG abnormalities such as spasticity (Lamontagne, et al., 2001), altered co-contraction (Lamontagne, et al., 2000), and muscle paresis (Lamontagne, et al., 2002) are higher in subjects with severe stroke, a cause-effect relationship of some of these abnormalities with poor locomotor performance (Detrembleur, et al., 2003) remains difficult to establish. The study of interlimb coordination during step-to-step transition in patients with stroke can give significant insights to help understand the low performance of stroke gait. Because restoration of gait is one of the major goals in stroke rehabilitation (Lindquist et al., 2007) and as step-to-step transition performance is important for global gait efficiency, understanding interlimb coordination is extremely beneficial for designing effective locomotor interventions.

2. METHODS

2.1 Subjects

Sixteen subjects (8 female; 8 male) who had suffered a stroke over 6 months ago and 22 healthy subjects (12 female; 10 male) participated in this study (Table 1).

Table 1: Mean and standard deviation (SD) values of age, height and weight of the healthy and stroke groups. The values of the self-selected walking speed adopted by each group are also indicated.

Variables	Stroke group	Control group	p-value
	Mean±SD	Mean±SD	
Age (years)	53.87±7.17	49.24±7.69	0.070
Height (m)	1.65±0.10	1.66±0.09	0.942
Body weight (Kg)	75.29±7.03	67.40±8.76	0.006
Self-selected gait speed ($m.s^{-1}$)	0.57±0.13	1.00±0.03	<0.001

For the patients with stroke, the mean time since the stroke until inclusion in this study was 26 months (SD=9). All subjects had suffered an ischemic stroke: 11 of them had suffered an infarction in their left hemisphere, whereas 5 had suffered an infarction in their right hemisphere. To be included, patients were required to: (1) have suffered a first-ever ischemic stroke involving the middle cerebral artery territory, as revealed by computed tomography, resulting in hemiparesis; (2) have a Fugl-Meyer (Assessment of Sensorimotor Recovery After Stroke scale) score in the motor subsection below 34; (3) have the ability to walk 10 meters, with close supervision if necessary, but without physical assistance, as judged by the supervising physiotherapist; (4) have provided written or verbal informed consent. Patients were excluded for one of the following reasons: (1) cognitive problems that could hinder communication and cooperation (assessed by the Mini-Mental State Examination); (2) history of orthopaedic or neurological (other than stroke) disorders known to affect walking performance; (3) history of stroke involving the brainstem or cerebellar areas; and (4) taking medication that could affect motor performance. Gait data of the patients with stroke were compared with data obtained from the 22 healthy control subjects. All control group subjects were sedentary and were selected according to the same exclusion criteria applied to the stroke group, however they were also excluded if they had suffered any neurological disorder. The study was approved by the local ethics committee and implemented according to the Declaration of Helsinki.

2.2 Instrumentation

The values of the vertical (F_z), anteroposterior (F_y) and mediolateral (F_x) components of the ground reaction force (GRF) were acquired using two force plates at a sampling rate of 1000 Hz (FP4060-10 and FP4060-08 models from Bertec Corporation (USA), connected to a Bertec AM 6300 amplifier and to a Biopac 16-bit analogical-digital converter, from BIOPAC Systems, Inc. (USA)).

The bilateral electromyographic activity (EMG) of Gastrocnemius Medialis (GM), Soleus (SOL), Tibialis Anterior (TA), Rectus Femoris (RF), Vastus Medialis (VM) and Biceps Femoris (BF) muscles was monitored using a bioPLUX research wireless signal acquisition system (Plux Ltda, Portugal). The signals were collected at a sampling frequency of 1,000 Hz and were pre-amplified at each electrode and then fed into a differential amplifier with an adjustable gain setting (25 - 500 Hz; common-mode rejection ratio (CMRR): 110 dB at 50 Hz, input impedance of 100 MΩ and gain of 1,000). Self-adhesive silver chloride EMG electrodes were used in a bipolar configuration and with a distance of 20 mm between detection surfaces' centre. The skin impedance was measured with an Electrode Impedance Checker (Noraxon USA, Inc.). The EMG and force platform signals were analysed with the Acqknowledge software (Biopac Systems, Inc., U.S.A). The gait timing was measured using a photovoltaic system (Brower Timing IRD-T175, Utah, USA). All subjects used the same shoe type, according to their size.

2.3 Procedures

2.3.1 Skin preparation and electrode placement

The skin surfaces of the selected mid-belly muscles and patella were prepared (shaved, dead skin cells and non-conductor elements were removed with alcohol and an abrasive pad) to reduce the electrical resistance to less than 5000 Ω, the EMG electrodes were placed according to anatomical references, and the reference electrode was placed on the patella.

2.3.2 Data acquisition

a) Kinetic and electromyographic data

The EMG and GRF data were acquired simultaneously during walking. Subjects walked using standard footwear over a 10 m walkway (Whitle, 2007) without using any assistive devices and/or orthotics. Two separate force plates were mounted in series near the midpoint of the walkway.

Before the data acquisition, sufficient time was given so that the participants become familiar with the experimental settings and they were allowed to walk over the walkway without any explicit instructions. In the meanwhile, we observed the starting point on the walkway from which they placed one foot on the first force plate (TRAIL) and the other on the second force plate (LEAD) according to their natural cadence. To ensure a low intra-group gait variability (Masani, et al., 2002; Sekiya, et al., 1997; Shiavi, et al., 1987a; Sousa & Tavares, 2012a), rate of energy expenditure (Detrembleur, et al., 2003) and muscle utilization ratios or levels of effort (Milot, et al., 2007; Requião, et al., 2005) similar between both groups the subjects walked at their self-selected speeds. All subjects performed three successful trials, where the feet had full contact with the plate. These were used for analysis to reduce the within-individual variability and increase statistical power (Mullineaux, et al., 2001).,One minute breaks were provided between trials.

2.3.3 Data processing

a) Kinetic parameters

The GRF data were low-pass filtered using a cutoff frequency of 8 Hz, with a fourth-order Butterworth filter by using a zero-phase lag (Winter, et al., 1990) normalised according to body weight. The stance phase was separated into 3 intervals to analyse impulse generation at various time points in the gait cycle: (1) DS after the heel strike of the paretic limb and before the non-paretic limb swing initiation (pre-swing), (2) MS-DS (push-off) and (3) DS after heel strike of the non-paretic limb and before the paretic limb swing initiation (pre-swing). The stance phase was defined as the interval where F_z presents a value equal to or higher than 7% of body weight (BW) (Cappellini, et al., 2006; Grasso, et al., 1998; Sousa & Tavares, 2012a); the DS corresponds to the time between the LEAD stance phase initiation until the initiation of the TRAIL swing phase; and the MS-DS was defined as the time between when F_y assumes the value zero (F_y=0) and the beginning of the second DS (Figure 1). Variables derived from F_y were time integrated to assess the braking (negative F_y) and propulsive (positive F_y) impulses. The percentage of the propulsion impulse generated by the TRAIL limb during the DS in relation to the LEAD limb was calculated by dividing the GRF impulse of the TRAIL limb by the sum of the GRF impulse of LEAD and TRAIL limbs.

b) Electromyographic activity

The EMG of the muscles of both limbs, analysed during the intervals selected to evaluate propulsive and braking impulse (Figure 1), were filtered using a zero-lag, second-order Butterworth filter with an effective band pass of 20-450 Hz, and the root mean square (RMS) was calculated. The EMG signals for each subject were normalised to the mean signal for each muscle over the entire gait cycle (Yang & Winter, 1984).

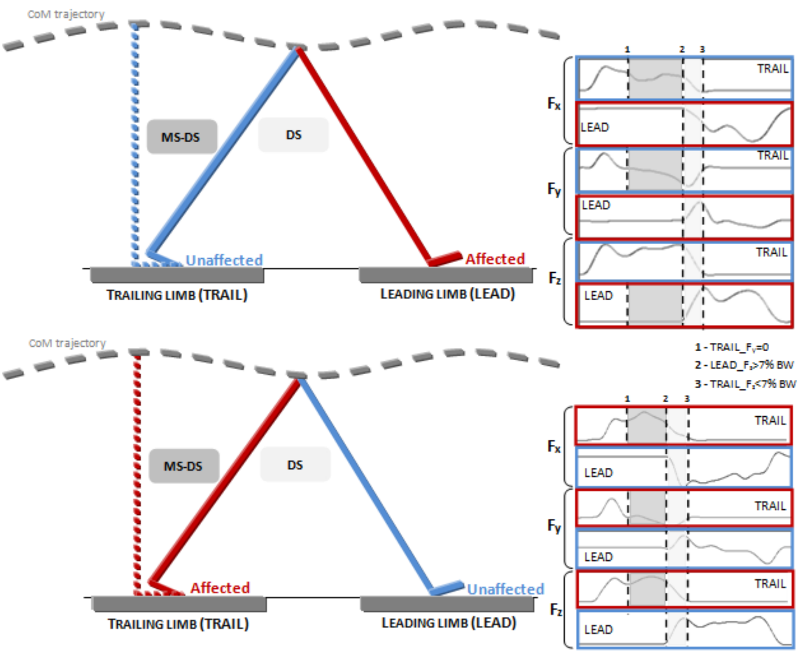

Figure 1: Representation of the intervals used to assess interlimb relation during the stance phase of walking in stroke subjects.

2.4 Data analysis

The acquired data was analysed using the Statistic Package Social Science (SPSS) software from *IBM* (USA). Spearman's and Pearson's correlation coefficient tests were used to assess the relation between paretic and non-paretic limbs in terms of EMG and propulsive/braking impulse, respectively. In healthy subjects the interlimb relation was analysed only in terms of propulsive/braking impulse, as interlimb relationships in terms of

EMG activity were analysed in our previous study (Sousa, Silva, et al., 2012b). The Paired Samples T-test was used to compare the EMG, propulsive and braking impulse levels and the relative propulsive contribution between paretic and non-paretic limbs, and the Independent Samples T-test was used to compare the same variables of the paretic and non-paretic limbs of the stroke group to those of the control group. The Wilcoxon and Mann-Whitney tests were used to compare the activity of the muscles not following a normal distribution (Shapiro-Wilk test and histogram analysis). To compare EMG and propulsive impulse levels between limbs of subjects with stroke and between stroke and control group in the three stance subphases, the Bonferroni correction was used to reduce type I error. Statistical significance was set at $p<0.05$.

3. RESULTS

3.1 Interlimb relation: EMG activity

Figure 2 shows the pattern of relative EMG activity observed in each limb of the stroke and control groups during DS and MS-DS. As depict in Figure 3, during DS higher values of the GM, SOL and BF of the non-paretic limb during heel strike led to lower VM values of the paretic limb during pre-swing, while higher values of SOL and RF of the non-paretic limb during MS-DS led to lower SOL and VM values of the paretic limb during heel strike. It was also interesting to note that the higher the VM activity of the paretic limb was during pre-swing, the higher the activities of the RF ($r=0.514$, $p=0.05$) and the GM ($r=0.539$, $p=0.038$) of the same limb were.

3.2 Interlimb relation: propulsive and braking impulse

Lower values of propulsive impulse (Figure 4) and of relative propulsive contribution (Figure 5) were found in the paretic limb of the stroke group during pre-swing. The control group presented significantly statistical correlations between the propulsive impulse of the TRAIL limb and the braking impulse of the LEAD limb during DS ($r=-0.568$, $p=0.004$) and during MS-DS and DS ($r=-0.512$, $p=0.011$). However, in the stroke group only the braking impulse of the non-paretic limb during heel strike influenced the propulsive impulse of the paretic limb at pre-swing during DS ($r=-0.639$, $p=0.010$).

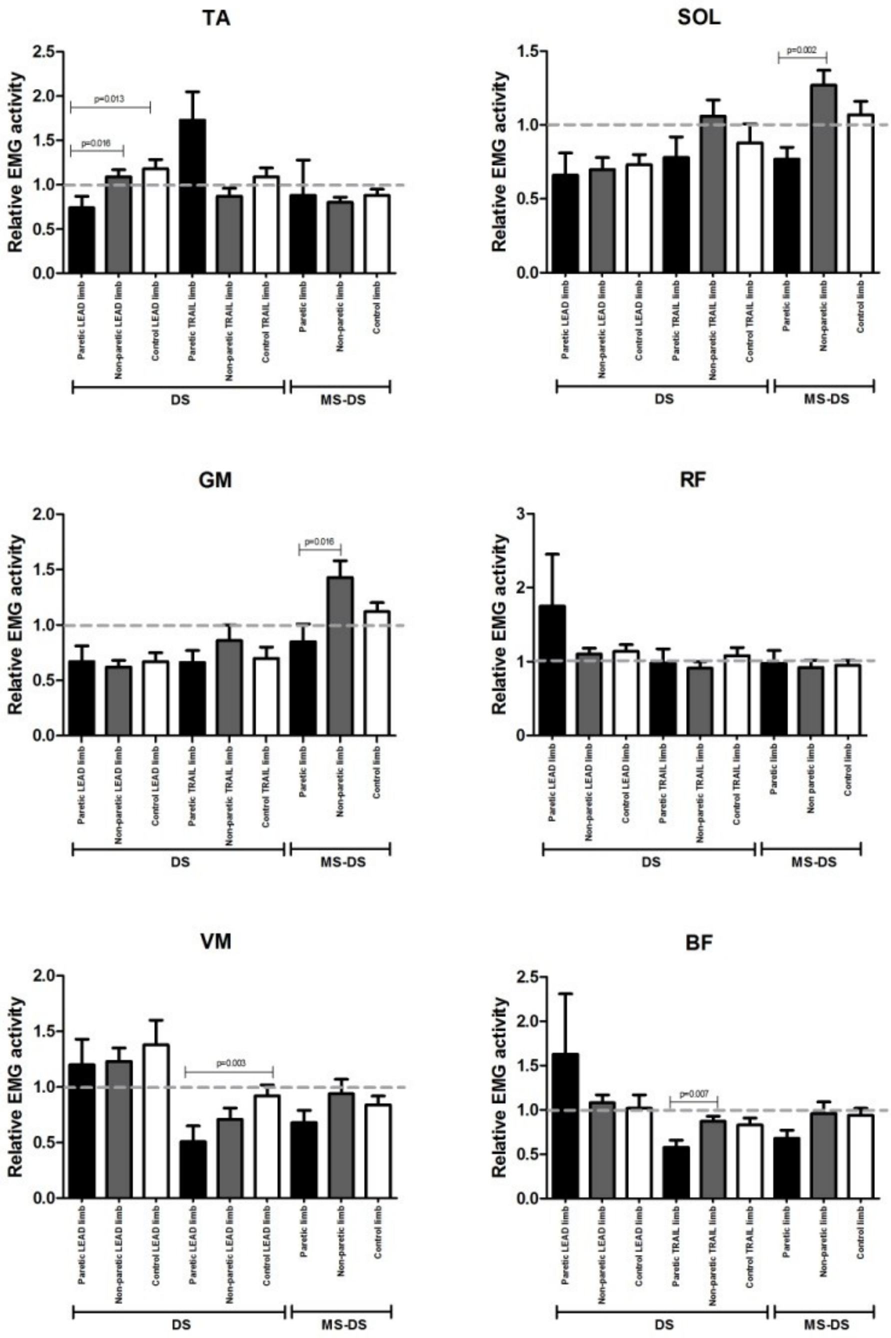

Figure 2: Mean (bars) and standard deviation (error bars) of RF, GM, TA, SOL, BF and VM EMG values observed in paretic and non-paretic limbs of subjects with stroke and healthy controls during DS and MS-DS (*significant correlation (p<0.05)). Values above the dashed line mean that the muscle activity was higher than the mean value during the stance phase, indicating the period where the muscle is more active.

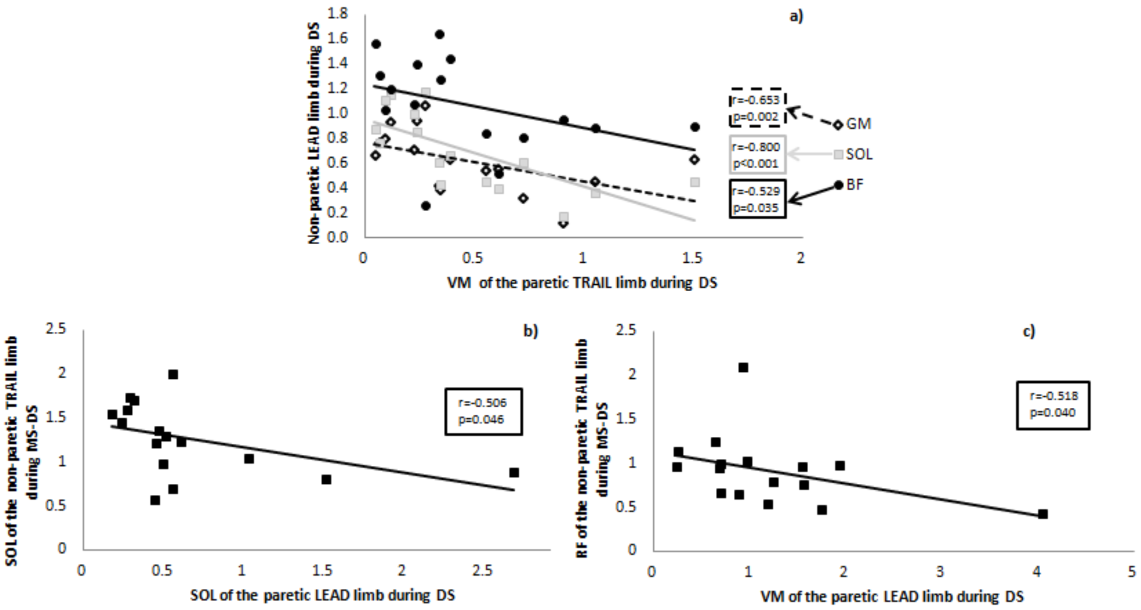

Figure 3: Mean (bars) and standard deviation (error bars) of mediolateral, anteroposterior and vertical impulse values observed in paretic and non-paretic limb of post-stroke subjects and healthy control during DS, DS-MS and MS-DS (*significant correlation (p<0.05)).

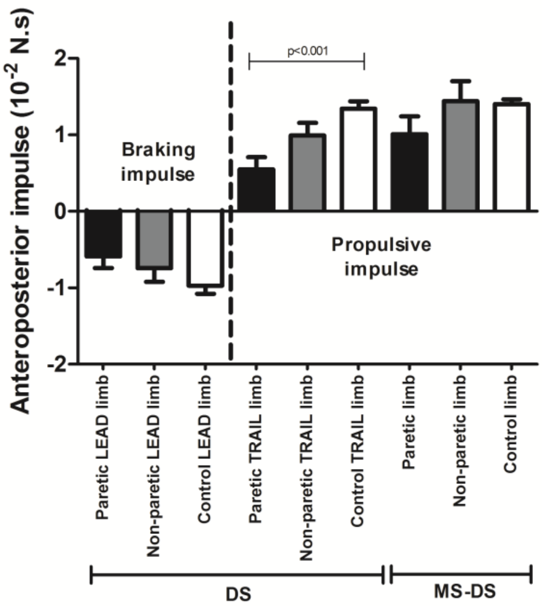

Figure 4: Mean (bars) and standard deviation (error bars) of propulsive and braking impulse observed in paretic and non-paretic limbs of subjects with stroke and healthy controls during DS and MS-DS (*significant correlation (p<0.05)).

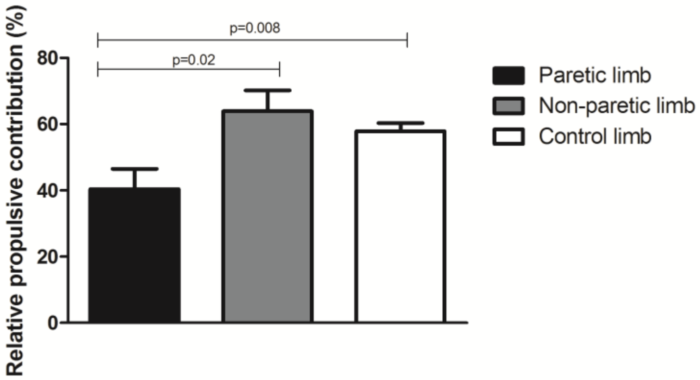

Figure 5: Mean (bars) and standard deviation (error bars) values of the percentage of TRAIL propulsive contribution of the paretic and non-paretic limbs of subjects with stroke and of TRAIL propulsion contribution in healthy controls during DS (*significant correlation ($p<0.05$)).

4. DISCUSSION

The participants of this study were asked to walk at a comfortable speed, and consequently different walking speeds were adopted by the subjects. The adoption of a low self-selected speeds has demonstrated that patients with stroke have an energy expenditure rate (Detrembleur, et al., 2003) and muscle utilization ratio or levels of effort (Milot, et al., 2007; Requião, et al., 2005) that are similar to those of healthy subjects walking at a comfortable speed. In this sense, it can be argued that the differences observed in self-selected speed did not interfere with our results. Also, the results obtained as to speed indicate that the impairment of the subjects with stroke can be classified as moderate (Perry, et al., 1995).

Recent works have demonstrated that each limb affects the strength of muscle activation and time-space behaviour of the other (Sousa, Silva, et al., 2012b; Stubbs, et al., 2009). The results of our study demonstrate that in patients with stroke the EMG and impulse levels of the TRAIL limb were related to the ones of the LEAD limb during DS, but only when the LEAD limb was the non-paretic limb and the TRAIL limb was the paretic limb. The higher levels of BF activity of the non-paretic limb were associated with lower levels of the VM activity of the paretic limb, which despite not developing an important role in this subphase (Figure 2) is positively correlated to the level of activity of the GM that contributes to swing initiation (Neptune, et al., 2001) and of the RF that accelerates the trunk forward (Neptune, Kautz, et al., 2004; Zajac, et al., 2003). Considering that the action of the BF muscle has been related to impact reduction during heel strike (Liu, et al., 2006; Sadeghi, et al., 2002; Whitle, 2007), the inverted indirect relation between the activity of this muscle and the GM and RF of the paretic limb during pre-swing is consistent with the inverted correlation observed between the braking impulse of the non-paretic limb and the propulsive impulse of the paretic limb. These results seem to demonstrate that the non-paretic limb would improve the coordination deficits of the paretic limb during DS because all its ‘appropriate’ sensorimotor information could be integrated by the nervous system and would contribute to a more appropriate pattern in the paretic limb. However, it should be noted that the SOL activity increases the horizontal energy of the trunk much more than the GM, especially in the late stance (McGowan et al., 2008; Neptune, et al., 2001), and no influence was exerted in this muscle by the non-paretic limb. Moreover, an inverted strong/moderate correlation was also observed between the SOL and GM of the non-paretic limb during heel strike and the VM of the paretic limb during pre-swing. Considering that the SOL and GM antagonist has an agonist role in impact reduction (Turns et al., 2007), it would be expected, according to the reciprocal inhibition mechanism, that higher SOL and GM values would be associated with higher VM levels. Some explanations can be proposed to interpret this non-functional interlimb relationship: First, excessive co-activation values of plantar flexor and dorsiflexor muscles have been demonstrated (Lamontagne, et al., 2002) as a consequence of ipsilaterally mediated effects from the neurological lesion (Shiavi, et al., 1987a; Silva, Sousa, Tavares, et al., 2012) and/or to an adaptation for poor stability during gait (Lamontagne, et al., 2000). However, no positive correlation was found between the activity of plantar flexors and the TA of the non-paretic limb during heel strike, and no differences were observed in the ankle muscle co-activation between the non-paretic limb of patients with stroke and control subjects (unpublished work). Another explanation could be related to recent evidence indicating that the spinal excitation from ankle dorsiflexors to knee extensors through group II is particularly enhanced during post-stroke in initial stance phase of walking, probably due to plastic adaptations in the descending control group (Achache et al., 2010).

A relationship between ankle dorsiflexors during heel strike and contra-lateral knee extensors can be hypothesised since most midlumbar interneurones which are recipient from group II input are influenced by afferent fibres from both ipsilateral and contra-lateral sides (Bajwa, et al., 1992). Taking into account the mechanism of reciprocal inhibition, the negative relation between the plantar flexors and the VM could be explained on the basis of the mechanism exposed. However, our results failed to demonstrate any inverted relationship between the plantar flexors and the TA. Altogether, these observations support the hypothesis of a possible agonist-antagonist deregulation related to the negative influence of the non-paretic limb on the paretic limb. Thus, future studies should analyse the neurophysiological mechanisms involved in the agonist-antagonist relationship during the stance phase of gait, particularly during heel strike.

Muscle activity of the paretic limb during heel strike was also influenced by non-paretic limb during the preceding MS-DS. The results indicate that higher SOL levels of the non-paretic limb during MS-DS could potentiate the TA activity of the paretic limb during heel strike. . This influence is close to the interlimb relationship observed in healthy subjects (Sousa, Silva, et al., 2012b). In fact, in healthy subjects higher activity of SOL during MS-DS has been shown to be associated to higher activity of muscles responsible for impact reduction (Sousa, Silva, et al., 2012b).

When one compares in a global manner the results of the patients with stroke with the healthy subjects (Sousa, Silva, et al., 2012b), it is evident that the TA, BF and VM muscles have an important role in contra-lateral limb activity in healthy subjects, while for the stroke patients SOL was the non-paretic limb muscle which consistently had more influence on the paretic limb, and the TA did not have any role in the interlimb relationship. This total lack of influence of the non-paretic limb on the TA of the paretic limb can be explained by the fact that this muscle depends more strongly on motor cortex input (Capaday et al., 1999; Yang & Gorassini, 2006). This input is disturbed in lesions at the internal capsule, through the affection of the corticospinal tract, which happened in the subjects with stroke that participated in our study. The role of the SOL muscle in mediating the interlimb relationship in stroke patients can be justified as it has a higher dependence on sensory inputs in relation to supraspinal control (Beres-Jones & Harkema, 2004; Harkema et al., 1997). However, the results here also demonstrated that the functional influence of the non-paretic limb SOL muscle on the paretic limb depends on its role on hand at the time. When this muscle acts as an agonist for movement, i.e., during push-off it promotes forward progression of the trunk (Neptune, et al., 2001)and exerts an influence on the homolog muscle of the paretic limb similar to healthy subjects (Sousa, Silva, et al., 2012b). But when its activity is more related to postural control, such as during heel strike, it exerts a biomechanically disadvantageous influence on the paretic limb considering the double inverted pendulum model and the interlimb relationship observed in healthy subjects (Sousa, Silva, et al., 2012b). These findings support the argument for the dysfunction of the ventral-medial system over the IPSI limb, which has also been hypothesised in other studies (Silva, Sousa, Pinheiro, Ferraz, et al., 2012; Silva, Sousa, Pinheiro, Tavares, et al., 2012; Silva, Sousa, Tavares, et al., 2012). Moreover it can be stated that dysfunction of ventral-medial system could be one of the causes for impaired interlimb coordination in stroke subjects. This hypothesis assumes a special importance considering that the stroke patients in this study presented lesions at the internal capsule, which can be associated to dysfunction of the cortico-reticular pathway (Drew, et al., 2004) responsible for ipsilateral postural control.

The EMG was selected to assess the level of individual muscle activity because no techniques or models are currently available that allow a valid quantification of individual muscle energy or muscle work *in vivo*. However, the adoption of this method presents limitations that should be noted. The mean normalisation method was selected to reduce the intersubject variability (Burden, et al., 2003) of muscle activity pattern during walking in stroke patients (Chau, et al., 2005; Hwang, et al., 2003; Kim & Eng, 2003) and to express possible interlimb relationships between the active muscles and the quiet muscles or between the active muscles of one limb and the quiet muscles of the contra-lateral limb. However, it should be noted that this method presents limitations when comparing the relative values of each muscle between stroke and healthy subjects (Figure 2).

According to Bowden et al., 2006, propulsive impulse provides a quantitative measure of the coordinated output of both lower limbs in stroke patients. The propulsive impulse of the paretic limb was lower than 50% (≈ 40%), which means that during the transition from pre-swing of the paretic limb to heel strike of the non-paretic limb the energy loss provoked by the non-paretic heel strike probably is not compensated by propulsion of the paretic limb decreasing the velocity of the centre of mass (Kuo, et al., 2005, 2007). Indeed, the paretic limb has been shown to produce significantly less mechanical work output than limbs of healthy subjects (Brown & Kautz, 1999). In stroke patients the relative propulsive impulse of the non-paretic limb exceeds the braking impulse (≈ 60%) of the paretic limb during heel strike and probably accelerates the centre of mass (Kuo, et al., 2005, 2007). It is important to note that in the control group the propulsive impulse of the TRAIL was also higher than 50%. In fact, previous studies in healthy subjects demonstrate that positive mechanical work of the TRAIL during DS exceeds the negative mechanical work performed by the LEAD (Sousa, Silva, et al., 2012b).

5. CONCLUSIONS

The results of the EMG and propulsive impulse demonstrate that the paretic limb presents lower performance in forward propulsion when compared with the non-paretic limb and the control group. Despite exerting an indirect functional influence on the activity of plantar flexors, the non-paretic limb exerted a dysfunctional influence during heel strike on the paretic limb during pre-swing. The characterization of influence of the non-paretic limb on the paretic limb was based on the interlimb relationship observed in healthy subjects (Sousa, Silva, et al., 2012b), the double-inverted pendulum model (Kuo, et al., 2005, 2007) and the role of individual muscles during the stance phases associated with step-to-step transition (Neptune, et al., 2001; Neptune, Kautz, et al., 2004). These findings seem to suggest that the lower performance of the paretic limb in forward propulsion is not only related to contra-lateral supraspinal damage, but also to the influence of the non-paretic limb in stance subphases of high postural control demand. These results backup arguments for considering an indirect impact of a postural control dysfunction of the IPSI limb on the performance and efficiency of gait in patients with stroke. Future works should be developed to explore this possibility as the lower performance and lower efficiency of gait in patients with stroke has been attributed until now only to alterations in the paretic limb. Therefore rehabilitation strategies should also give special attention to the non-paretic limb to potentiate the activity of the paretic limb in stroke patients whose subcortical structures in the medial cerebral artery territory, such as in the internal capsule, have been affected. Specifically, the results obtained suggest that improving postural control of the non-paretic limb could have positive effects on the interlimb coordination during step-to-step transition and consequently on walking performance.

PART B – *ARTICLE VI*

Influence of wearing an unstable shoe on thigh and leg muscle activity and venous response

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ABSTRACT

Purpose: To quantify the effect of unstable shoe wearing on muscle activity and haemodynamic response during standing. *Methods:* Thirty volunteers were divided into 2 groups: the experimental group wore an unstable shoe for 8 weeks, while the control group used a conventional shoe for the same period. Muscle activity of the medial gastrocnemius, tibialis anterior, rectus femoris and biceps femoris and venous circulation were assessed in quiet standing with the unstable shoe and barefoot. *Results:* In the first measurement there was an increase in medial gastrocnemius activity in all volunteers while wearing the unstable shoe. On the other hand, after wearing the unstable shoe for eight weeks these differences were not verified. Venous return increased in subjects wearing the unstable shoe before and after training. *Conclusions:* The unstable shoe produced changes in electromyographic characteristics which were advantageous for venous circulation even after training accommodation by the neuromuscular system.

Keywords: *Electromyography; Haemodynamics; Muscle pumps*

1. INTRODUCTION

The primary function of venous circulation is to return blood to the heart. Effective venous return requires the interaction of a central pump, a pressure gradient, a peripheral venous pump, and competent venous valves to overcome the forces of gravity (Araki et al., 1994; Ludbrook, 1966; Meissner et al., 2007). Depending on activity and posture, 60-80% of the resting blood volume resides in the venous system (Katz et al., 1994) and in the upright but motionless individual the hydrostatic pressure is higher (Meissner, et al., 2007). As the thin-walled veins readily distend with relatively small increases in transmural pressure (Rothe, 1983), compensatory mechanisms must be present to prevent the blood from pooling in the extremities and aid in the return of blood to the heart. Indeed the peripheral pump may “drive” the circulation during exercise (Rowland, 2001).

The muscle pumps of the lower limb include those of the foot, calf and thigh. Among these, the calf muscle pump is the most important, as it is the most efficient, has the largest capacitance and generates higher pressures (Katz, et al., 1994). The normal limb has a calf volume ranging from 1500-3000 cm^3, a venous volume of 100-150 cm^3, and ejects over 40 to 60% of the venous volume with a single contraction (Araki, et al., 1994; Stewart et al., 2004). Evidence suggests that dynamic exercise produces increased blood flow when compared to continuous isometric exercise (Laughlin & Armstrong, 1985). During dynamic exercise, the muscle pump plays an important role in initial increase and maintenance of blood flow (Laughlin & Schrage, 1999) as blood flow increases between contractions, even for low intensity ones (Radegran, 1997). With walking, the limb venous pressure is reduced by approximately 78 mmHg within 7-12 steps (Pollack & Wood, 1949). Similar pressure changes are observed during standing with ankle flexion or heel raising, with weight transferred to the forefoot (Nicolaides et al., 1993; Pollack & Wood, 1949).

Chronic venous insufficiency explains those manifestations of venous disease resulting from ambulatory venous hypertension, which is associated with failure of the lower extremity muscle pumps due to outflow obstruction, musculo-fascial weakness, loss of joint motion or valvular failure (Araki, et al., 1994; Nicolaides, et al., 1993; Stewart, et al., 2004). Efficient peripheral pumps may compensate for some degree of reflux and obstruction and prevent chronic venous insufficiency symptoms (Padberg et al., 2004; Plate et al., 1986). It has been demonstrated that calf muscle strengthening exercises restore the pumping ability of the calf muscle and improve the haemodynamic performance in limbs with active ulceration subsequent to severe venous valvular and calf muscle pump impairment (Padberg, et al., 2004).

It has been suggested that balance training devices, such as wobble-boards or unstable surfaces, can significantly improve ankle and knee muscle strength and proprioception (Waddington & Adams, 2004; Waddington et al., 2000; Wester et al., 1996). Previous experiments dealt with changes in gait characteristics, posturography and electromyographic activity (EMG) of several lower extremity muscles (gastrocnemius, tibialis anterior, vastus lateralis and medialis, rectus femoris, and semitendinosus) in healthy subjects (Nigg, Hintzen, et al., 2006; Romkes, et al., 2006; Stewart et al., 2007), in children with development disabilities (Ramstrand, et al., 2008), in women aged over 55 years (Ramstrand, et al., 2010), and in patients suffering from osteoarthritis (Nigg, Emery, et al., 2006) in response to unstable shoe wearing.

Therefore, the purpose of this study was to quantify the effect of wearing an unstable shoe on muscle activity and haemodynamic response in lower extremities during standing before and after training intervention. Specifically, the purposes were:

- to evaluate the immediate effect of unstable shoe wearing on EMG activity of medial gastrocnemius (MG), tibialis anterior (TA), biceps femoris (BF) and rectus femoris (RF) muscles during standing, to provide evidence for changes in muscle activation;

- to analyse the influence of 8 weeks of unstable shoe wearing on EMG activity, to provide evidence for changes in muscle activation;

- to quantify the immediate effect of unstable shoe wearing on lower limb venous circulation;

- to quantify the influence of 8 weeks of unstable shoe wearing on lower limb venous circulation.

In all situations the values obtained before and after 8 weeks of intervention with the unstable shoe were compared to standard measures obtained during barefoot standing.

The following hypotheses were tested:

EMG activity

H1 - During quiet standing, the EMG intensity of all muscles analysed is higher for the unstable shoe condition compared to barefoot standing before and after the unstable shoe intervention.

H2 - During quiet standing, the EMG intensity levels are lower after 8 weeks of unstable shoe wearing.

Lower limb venous circulation

H3 - During quiet standing, lower limb venous circulation is higher for the unstable shoe condition compared to barefoot standing before and after the unstable shoe intervention.

H4 - During quiet standing, lower limb venous circulation is lower after 8 weeks of unstable shoe wearing.

2. METHODS

2.1 Subjects

Thirty healthy female individuals between the age of 20 and 50 years, distributed in 2 groups matching in age, weight and height were included. The study excluded subjects presenting one or more of the following aspects: (1) recent osteoarticular or musculotendinous injury of the lower limb; (2) background and signs of neurological dysfunction which could affect lower limb motor performance, sensory afferences and balance; (3) history of surgery in lower extremities; (4) pain in lower extremities and trunk for the past 12 months; (5) taking medication; (6) balance disorders and visual deficits; and (7) individuals who had used unstable footwear prior to the study. The same exclusion criteria were adopted for both groups.

The study included individuals whose professional occupation was mainly executed while standing statically. The experimental group included 14 individuals (age = 34.6 ± 7.7 years, height = 1.6 ± 0.1 m, weight = 65.3 ± 9.6 kg; mean ± SD) and the control group included 16 individuals (age = 34.9 ± 8.0 years, height = 1.6 ± 0.1 m, weight = 61.1 ± 6.3 kg; mean ± SD). In both groups, the dominant lower limb was the right. The study was conducted according to the institution ethical norms and conformed to the Declaration of Helsinki, with informed consent obtained from all participants.

2.2 Instrumentation

The EMG activity of the MG, TA, RF and BF was monitored using the MP 150 Workstation model from Biopac Systems, Inc. (USA), bipolar steel surface electrodes, spaced 20 mm apart, and a ground electrode (Biopac Systems, Inc.). EMG signals during quiet standing show excellent repeatability (Lehman, 2002). Skin impedance was measured with an Electrode Impedance Checker (Noraxon USA, Inc.).

The cross-sectional area and the venous velocity of the common femoral (CFV) and popliteal (PV) veins were determined using an Acuson CV 70 duplex ultrasound unit (Siemens Medical Solutions, USA), with a 5-10 MHz linear array probe.

2.3 Procedures

2.3.1 Skin preparation and electrode placement

We have prepared the subjects' lower limbs to reduce electrical resistance to less than 5000 Ω (Basmajian & De Luca, 1985) by (1) shaving the skin surface of the muscle belly area; (2) removing dead cells with alcohol; and (3) removing non-conductor elements between electrode and muscle with abrasive pad (Hermens, et al., 2000).

Electrodes were placed at the centre of the muscle belly of the MG, TA, RF and BF. The reference electrode was placed at the centre of the patella. To avoid movement and to ensure homogeneous and constant pressure, the electrodes were fixed to the skin with adhesive tape (Hermens, et al., 2000). We waited 5 minutes after electrode placement to begin measurements as evidence suggest that there is a reduction of 20-30% in impedance values during the first 5 minutes after electrode placement (Vredenbregt & Rau, 1973).

2.3.2 Data collection

In the experimental group, the EMG and haemodynamic data were collected in 2 conditions: (1) prior to using the unstable shoe and (2) after wearing it for a period of 8 weeks. Subjects in the control group were also assessed at 2 moments separated by 8 weeks, but using a conventional shoe between them. In both groups and in all assessments the variables evaluated were monitored under 2 conditions: (1) upright barefoot standing and (2) upright standing wearing the unstable shoe (Figure 1). Trials were randomised to reduce the order effect, which can be caused by previous muscle activation or learning. Measurements were performed on the dominant limb, which was the right limb. Before data acquisition, all subjects underwent an instruction session by a qualified instructor who explained how to use the unstable test shoe, followed by approximately 10 minutes of walking, until the instructor felt they walked properly and were comfortable using the shoe (Nigg, Hintzen, et al., 2006).

Figure 1: Unstable shoe model used in this study: The MBT shoe has a rounded sole in the antero-posterior direction, thus providing an unstable base.

All individuals were asked to remain comfortably standing, with the support base aligned at shoulder width, keeping their arms by their sides. To ensure optimum test-retest reliability, they were also given a target 2 meters away at eye level on which to focus during the 30 seconds of data acquisition. Data acquisition initiated 3 seconds after the beginning of the testing procedure and was done in a total of 3 trials.

EMG signals were acquired at a sample rate of 1000 Hz, then digitised and stored on a computer for subsequent analysis by the Acqknowledge software (Biopac Systems, Inc.

USA). Signals were pre-amplified at the electrode site and then fed into a differential amplifier with adjustable gain setting (12-500 Hz; Common Mode Rejection Ratio (CMRR): 95 dB at 60 Hz and input impedance of 100 MΩ). The gain range used was 1000. A 30 second window of EMG signal was used for analysis, and signals were band-pass filtered between 20 and 450 Hz. This window of raw EMG activity was processed using the Root Mean Square (RMS) procedure. The mean of the RMS was normalised in relation to a maximal isometric contraction, performed after a warm-up consisting of 3 submaximal isometric contractions (Lehman & McGill, 1999). TA and MG maximal isometric contractions (MIC) were measured with the ankle in neutral position. MIC for the BF and RF were measured with the knee at 90º. For all muscles manual resistance was applied.

The cross-sectional area and venous velocity were monitored in the CFV and PV. PV measurements were examined directly behind the knee joint and CFV measurements were taken approximately 2 cm above the saphenofemoral junction.

Three separate measurements of venous velocity were obtained and the mean values computed. As the peripheral blood flow is affected by respiratory manoeuvres (Tortora & Anagnostakos, 1990; Willeput et al., 1984), subjects were asked to maintain a stable respiratory pattern during data acquisition. The maintenance of constant temperature conditions was also provided (Henry & Gauer, 1950). The volume flow rate (Q) in the blood vessel was calculated by multiplying the cross-sectional area of the blood vessel (A) by the mean velocity (v) of the blood within it (Brown et al., 1989):

$$Q = v \times A$$

Following an initial evaluation, subjects in the experimental group were given a pair of the unstable test shoe. They were instructed to wear them as much as possible, for at least 8 hours a day, 5 days a week, for 8 weeks, as it has been demonstrated that wearing unstable shoes during a period of 8 weeks induces improvements on postural control (Ramstrand, et al., 2008; Ramstrand, et al., 2010). Also, there is evidence that 6 weeks of unstable wearing induces training effects (Kalin & Segesser, 2004; Nigg, Hintzen, et al., 2006; Romkes, et al., 2006; Vernon et al., 2004). Subjects were given a guide on how to use the shoes and participants in the control group were asked to continue their normal activities and not begin any new exercise regime. The second evaluation was performed 8 weeks after the first, using the same protocol. The experimental group wore the unstable shoe only during working time (at least 8h per day). As all subjects were hairdressers, they were most of the time in upright standing.

2.4 Statistics

Statistical analysis was processed with Statistic Package Social Science (SPSS) from IBM Company (USA). The sample was characterised by descriptive statistics.

Differences in lower extremity venous return and EMG activity between the first and second evaluation were analysed using the Paired Samples t-test and the Wilcoxon test, respectively, as EMG values did not follow a normal distribution. To analyse differences between groups, the Independent Samples t-test was used to compare venous return measurements and the equivalent non-parametric test. The Mann-Whitney U test was used to compare EMG measurements. Differences between measurements with the unstable test shoe and barefoot were analysed using the Wilcoxon test for the EMG measurements, as these values did not follow a normal distribution, and the equivalent parametric test, the Paired Samples t-test, for the venous flow. A 0.01 significance level was used for inferential analysis.

3. RESULTS

3.1 Influence of unstable footwear on muscle activity

Comparing the mean of EMG activity of each muscle between the experimental and control groups, it can be stated that there were no significant differences in TA, MG, BF and RF muscles in the first and the second evaluation (Figure 2). There were no significant differences in muscle activity level in the experimental group, before and after 8 weeks of wearing the unstable shoe, and in the control group, before and after 8 weeks of conventional shoe wearing (Figure 2).

In the first measurement, both groups presented significantly higher MG activity while wearing the unstable shoe when compared to barefoot (experimental group: $p=0.006$; control group: $p=0.009$), with no significant differences for the other muscles studied (Figure 2). In the second measurement, the experimental group showed no statistically significant differences in MG, TA, BF and RF activity between the 2 evaluated conditions (Figure 2). In the control group, both before and after the 8-week period, MG activity was higher when using the unstable shoe (p=0.007), while all other muscles studied showed no significant differences (Figure 2).

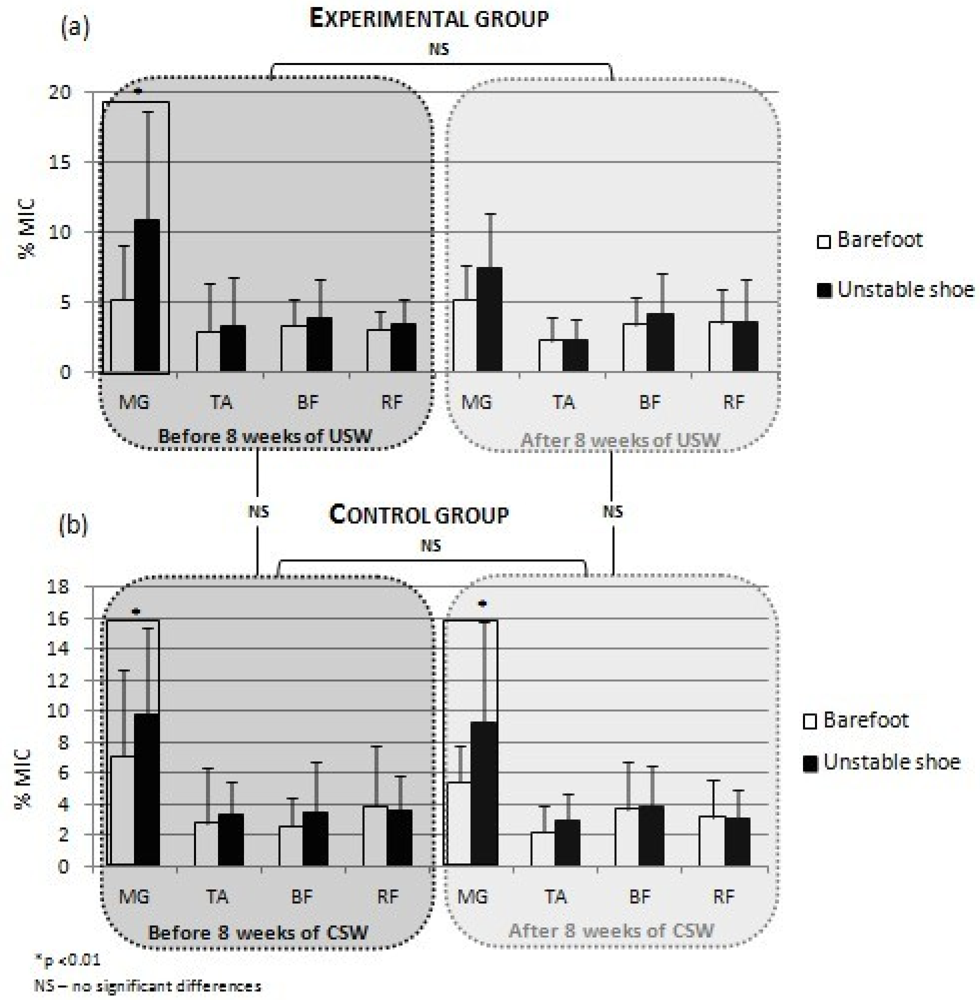

Figure 2: Representation of values for mean (bars) and standard deviation (error bars) of TA, MG, RF and BF muscles activity (% MIC) during standing with and without the unstable shoe before and after 8 weeks of unstable shoe wearing (USW) by the experimental group (a) and the same period of conventional shoe wearing (CSW) by the control group (b). The Wilcoxon test was used to compare EMG activity obtained with and without the unstable shoe, the values obtained before and after the 8-week period and the values obtained by the experimental group and the control group.

3.2 Influence of unstable footwear on venous return

Comparing the mean of flow rate at CFV and PV between the experimental and control groups, it can be stated that there were no significant differences between the control group and the experimental group for both the first and second evaluations (Figure 3).

A comparison between measurements taken with and without the unstable shoe shows a higher level of venous return with the unstable shoe in both groups during the first and second evaluation in PV (experimental group: p=0.006 and p=0.002, respectively; control group: p=0.004 and p=0.001, respectively) and CFV (experimental group: p=0.002 (for both situations); control group: p=0.001 (for both situations)) (Figure 3). As to the influence of 8 weeks of unstable shoe wearing, there were no differences between the first and second evaluation, both with and without the unstable shoe (Figure 3). The same was verified in subjects that wore conventional footwear (Figure 3).

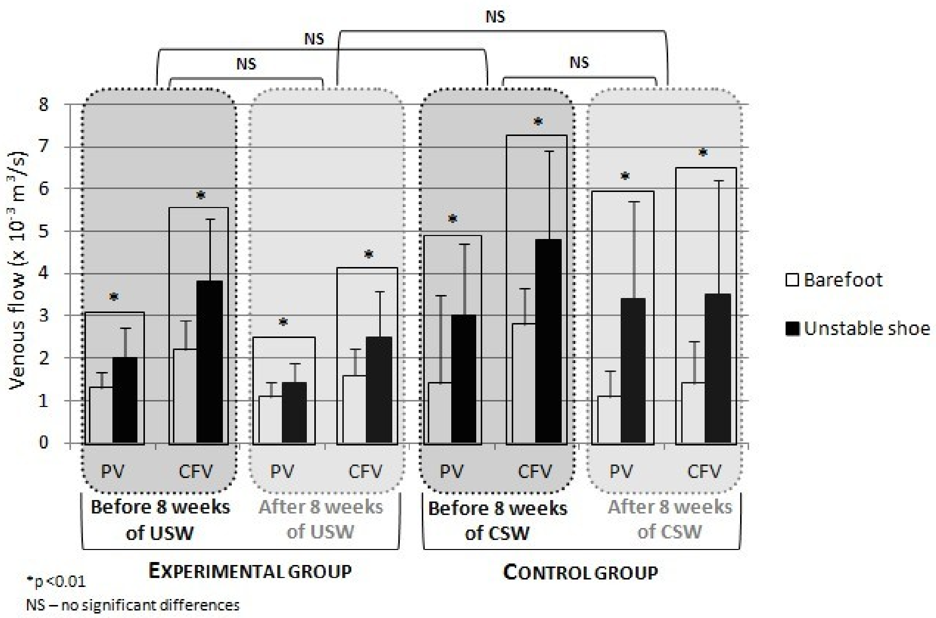

Figure 3: Representation of mean and standard deviation values of venous circulation at CFV and PV during standing with and without the unstable shoe before and after 8 weeks of unstable shoe wearing (USW) by the experimental group and 8 weeks of conventional shoe wearing (CSW) by the control group. The paired samples T-test was used to compare venous circulation obtained with and without the unstable shoe, before and after the 8-week period and between the experimental group and the control group.

4. DISCUSSION

In upright standing small postural adjustments occur, mainly at the ankle (one of several possible balancing strategies), and these adjustments are accompanied by small fluctuations in the activity and muscle length of plantar flexors (Loram, et al., 2005b), resulting in centre of mass (CoM) displacements (Gatev, et al., 1999). The results of this study show that using an unstable shoe (versus barefoot) leads to increased MG activity. In the study of Romkes et al. (2006) it has been demonstrated that using an unstable shoe changes movement patterns during gait, especially at the ankle, and increases muscle activity as well. It has also been shown that accommodations to a rockered sole during running occur only at the ankle (Boyer & Andriacchi, 2009). It seems that wearing an unstable shoe leads to changes in the ankle control pattern in a variety of activities. According to Ivanenko, Levik et al. (1997), when standing on a rocking support (seesaw), the CoM deviation is accompanied by changes in ankle movement pattern and plantar pressure distribution, which are compensated by gastrocnemius muscle activation as in this condition, instead of moving the CoM, subjects shift the point of contact of the rocking platform with the ground under the CoM. Nigg, Hintzen et al. (2006) reported an increase only for the TA during standing with the unstable shoe, when compared to the conventional shoe. Based on its construction, the unstable shoe used in this study forced the user to land more towards the midfoot. There is evidence that standing in unstable footwear leads to increased plantar flexion at the ankle joint, which corroborates the increased MG activity observed in this study (New & Pearce, 2007).

The experimental group results show that after 8 weeks of unstable shoe wearing, the MG activity with the unstable test shoe was not significantly different from the values obtained in the barefoot measurement, as verified before training. The design of unstable footwear used in the present study (MBT) is based on observations of the Masai tribe, who are not accustomed to wearing shoes. This design recreates natural uneven walking surfaces to reduce problems caused by today's rigid soled shoes and hard ground. The adaptation of the human biological system for movement control (Ferrel et al., 2000) includes changes in the response of neural receptors (Theunissen et al., 2000) and changes in the function of central and autonomous nervous systems (LeBlanc et al., 1975; Pia, 1985). Exercises repeated daily or weekly can improve postural control (Hu & Woollacott, 1994) and generate structural and functional adaptations in the neuromuscular system (Hakkinen et al., 1996). Our results as to the MG corroborate the idea that wearing an unstable shoe leads to neuromuscular system functional adaptations. Results obtained in the control group reinforce that differences between conditions in the first and second evaluations in the experimental group resulted from wearing the unstable shoe for 8 weeks. According to Ramstrand, Thuesen et al. (2010), reactive balance can be improved by prolonged and regular use of shoes incorporating an unstable sole construction. Standing with unstable shoes effectively activates extrinsic foot muscles and can have implications for strengthening and conditioning these muscles, as postural sway while standing with unstable shoes also decreases over a 6-week accommodation period (Landry, et al., 2010). Although the triceps surae is involved in plantar flexor activity, the gastrocnemius muscle seems to play a central role in the phasic control of balance (Borg et al., 2007). Results obtained by Gatev et al., 1999, showed that there is a significant statistical correlation between gastrocnemius activity and the position of spontaneous body sway, which was measured as the CoM position. This supports the notion that active torque is provided by gastrocnemius muscle contractions in response to body sway.

As to venous return, the results of this study show an increase in both PV and CFV measurements made while wearing the unstable shoe. This increase was observed in both groups and for both veins. Dynamic exercise causes higher and less heterogeneous blood flow than intermittent isometric exercise at the same exercise intensity (Laaksonen et al., 2002). During exercise the contraction rhythm of peripheral skeletal muscles results in the compression of intramuscular veins, granting the venous blood a considerable amount of kinetic energy which facilitates its return to the heart (Stewart, et al., 2004). The results of this study show that wearing an unstable shoe led to increased MG activity, which can lead us to think that venous return variations were more associated to MG EMG activation. Instantaneous changes in surface EMG amplitude may provide a good estimate of intramuscular pressure changes during the rising part of isometric, but also of concentric, voluntary contractions (Maton et al., 2006). During contraction, the gastrocnemius and soleus muscles drive blood into large capacity PV and CFV. Although thigh veins are surrounded by muscle, the contribution of thigh muscle contraction to venous return is minimal when compared to the calf muscle pump (Ludbrook, 1966).

During quiet standing, measurement of low intrinsic ankle stiffness (Loram & Lakie, 2002a), analysis of ballistic character of sways (Loram & Lakie, 2002b) and investigations of balance in an analogous task using a weak spring (Lakie, et al., 2003) provide increasing evidence that intermittent ballistic-like adjustments in muscle length (Loram & Lakie, 2002b) may be responsible for the apparently random sway pattern that is seen in quiet standing. As reported by Nigg, Hintzen et al. (2006), visual control of EMG signals (without signal processing and statistical analysis) showed more variation in measurements with the unstable shoe. Taking this into account, together with the fact that dynamic muscle contractions are advantageous to venous circulation, it would be interesting to investigate how the use of an unstable shoe affects muscle activity and muscle length time variation.

It is important to note that, although MG activity with the unstable shoe did not differ from barefoot measurements after 8 weeks of training in the experimental group, venous return did not decrease when compared to measurements made before the training period (Figure 3). Moreover, measurements made with the unstable test shoe after training revealed significantly higher venous return than barefoot measurements. According to Ivanenko, Levik et al. (1997), during overground standing the triceps surae muscles generally work in an eccentric mode of contraction, and on the seesaw in a concentric one. Unstable shoe sole configuration is similar to the seesaw used in (Ivanenko, et al., 1997), and therefore it can be assumed that it also favours concentric activity. These findings may explain why, despite MG neuromuscular adaptation, the venous flow remained higher while wearing the unstable test shoe, as most of the venous return occurs during the concentric phase of contraction (Hogan et al., 2003). RMS analysis shows that EMG activity may be related to increased venous flow. Nevertheless, future studies focusing a temporal analysis, could help understanding the influence of wearing unstable shoes in time variation of muscle parameters and its impact on venous flow to confirm our results.

It has been demonstrated that balance training improves postural control performance both in healthy subjects (Heitkamp, et al., 2001) and in injured individuals (Mattacola & Lloyd, 1997). Taking into account that patients with venous disease show weak calf muscle strength (Yang et al., 1999), it would be important, in future studies, to analyse the influence of using an unstable shoe on calf muscle activity and venous flow in patients with venous disease.

5. Conclusions

The findings of this study show that wearing an unstable shoe leads to a short-term increase in MG activity. However, after 8 weeks of unstable shoe wearing the activity of this muscle with unstable shoe was more close to the one obtained in the barefoot condition. As to venous return, results show that wearing an unstable shoe leads to increased venous return in PV and CFV and that this increase was maintained after 8 weeks of using the unstable shoe.

In summary, using an unstable shoe produced changes in EMG characteristics during upright standing which are advantageous for venous circulation even after training adaptation by the neuromuscular system.

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PART B – *ARTICLE VII*

The influence of wearing unstable shoes on upright standing postural control in prolonged standing workers

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ABSTRACT

Objective: To study the influence of prolonged wearing of unstable shoes (USW) on standing postural control in prolonged standing workers. *Methods:* Stabilometry parameters related to centre of pressure (CoP), rambling (RM) and trembling (TR) were assessed and total agonist/antagonist muscle activity, co-contraction index (CCI) and reciprocal activation (R) were calculated using electromyography. *Results:* USW led to: (1) decreased root mean square (RMS) and displacement and increased mean velocity (MV) of RM, (2) increased TR displacement and decreased TR RMS. No differences were observed in CoP related values and in the synergy between agonist and antagonist muscles. *Conclusions:* USW led to increased effectiveness and performance of supraspinal processes and of spinal reflexes and/or in the intrinsic mechanical properties of muscles and joints. The instability provided by USW in standing postural control remains even after adaptation by the postural control system.

Keywords: Stabilometry; Co-contraction index; Reciprocal activation; Postural control performance; Unstable support

1. INTRODUCTION

The support surface type has a relevant impact over postural control in humans (Dietz, et al., 1980; Gantchev & Dimitrova, 1996; Gavrilenko, et al., 1995; Ivanenko, et al., 1999). When standing on an unstable support the new postural requirements lead to postural control reorganisation through increased central drive (Gavrilenko, et al., 1995; Ivanenko, et al., 1999) associated with augmented gamamotoneuron activity leading to higher sensitivity of the muscle spindles (Dietz, et al., 1980; Gorassini, et al., 1993; Prochazka, 2010; Ribot-Ciscar, et al., 2000), changes in synergies between antagonist and agonist muscles (Dietz, et al., 1980) and increased anticipatory postural control adjustments (Arruin, et al., 1998; Gantchev & Dimitrova, 1996; Nardone & Schieppati, 1988; Nouillot, et al., 1992). Based on this, it can be argued that, depending on the degree, the instability provided by the unstable support condition would have positive effects over the postural control. Despite this possibility, the effect of unstable support conditions has been explored mainly at the immediate level or in balance training exercises. Considering the great adaptation of the CNS (Winter, 1984) in response to changing task and environment demands (Shumway-Cook & Woolacott, 2007), further investigation is required regarding the long-term influence of changes in afferent information during daily activities that could be beneficial to postural control. Recently, manufacturers have introduced new shoe designs to feature unstable conditions (Masai Barefoot Technology, MBT, USA) inducing neuromuscular training stimuli, similar to traditional balance training, in normal daily activities. However, divergence exists as to the benefits from wearing this kind of shoes on postural control. Previous research has demonstrated that wearing this kind of unstable shoe regularly leads to changes in muscle activity level, mainly at the ankle joint, during upright standing (Sousa, Tavares, Macedo, et al., 2012) and to decreased CoP excursion in young subjects (Landry, et al., 2010), although no changes have been observed on mean velocity of CoP in mid-aged women (Ramstrand, et al., 2010) neither in CoP excursion in one-leg stance in young subjects (Turbanski, et al., 2011). This divergence could result from the few parameters analysed, as a set of measures is required to detect differences in postural control (Pavol, 2005).

Upright stance is associated with a process of continuous small body deviations countered by corrective torques, generating a pattern known as spontaneous body sway. Involving a complex sensorimotor control system, upright postural control can be evaluated based on measurements of body segment displacement, muscle activity and displacement and motion patterns of the centre of mass (CoM) and the centre of pressure (CoP) (Balasubramaniam & Wing, 2002).

From a biomechanical perspective, a number of parameters derived from the CoP migration have been often used to characterise postural control and to evaluate postural performance (Bennell & Goldie, 1994; Collins & De Luca, 1993; Kinzey, et al., 1997; Maurer & Peterka, 2005). This is because CoP migration represents the summed up effect of mechanical muscle properties and of a number of different neuromuscular components whose characteristics are strongly dependent on the main inputs that control postural stability (Baratto, et al., 2002; Maurer & Peterka, 2005; Winter, 1995). However, CoP measures only represent the control variable acting to compensate CoM displacement (the controlled variable) (Morasso, et al., 1999). The importance of CoM measurements in association with CoP measurements is that the difference between the two variables is proportional to the horizontal acceleration of the CoM representing the "error" signal in the balance control system (Winter, 1995). According to Zatsiorsky, 1999, the nature of postural sway is the result of a moving reference point (rambling, RM). This moving point is related to the supraspinal process and constitutes a reference about which the body oscillates (trembling, TR) through the action of spinal reflexes and changes in the intrinsic mechanical properties of muscles and joints (Zatsiorsky & Duarte, 1999). The decomposition technique of CoP time series proposed by authors to assess RM and TR has been demonstrated to provide a very good estimate for both components (Lafond, et al., 2004).

Considering the aforementioned, the main purpose of this study was to analyse the influence of USW on upright standing postural control. More explicitly, the purposes were to evaluate the effect of wearing the unstable shoes depicted in Figure 1 on: 1) CoP displacement patterns, 2) CoP and CoM inter-relation through RM and TR components, 3) total agonist and antagonist muscle activity, and 4) agonist-antagonist muscle relation. To the best of our knowledge no previous study addressed the influence of USW on CoP and CoM interrelation or muscle synergies during quiet standing. However, based on previous research concerning the effect of unstable support conditions (Gavrilenko, et al., 1995; Ivanenko, et al., 1999) and on the effect of USW at individual muscle level (Sousa, Tavares, Macedo, et al., 2012), we hypothesised that the instability provided by USW would lead to higher performance of the postural control system. Specifically we hypothesised that USW would lead to higher values of CoP migration, higher CCI and lower R values at ankle and muscle group levels, higher total agonist muscle activity and higher TR performance.

The mechanisms involved in upright human postural control in response to USW constitute an important issue, not only in the motor control domain but also from an ergonomic perspective, as postural control changes associated with USW could reduce the negative outcomes that have been associated with prolonged upright standing (Brantingham et al., 1970; Macfarlane et al., 1997). Our previous study demonstrated that muscle activity adaptations associated to USW have positive effects on venous circulation during upright standing (Sousa, Tavares, Macedo, et al., 2012).

Figure 1: Unstable shoe model used in this study: The MBT shoe has a rounded sole in the antero-posterior direction, thus providing an unstable base.

2. METHODS

2.1 Subjects

The study included healthy female participants whose professional occupation requires prolonged standing positions, divided into two groups: the experimental group included 14 individuals (age = 34.6 ± 7.7 years, height = 1.59 ± 0.06 m, weight = 65.3 ± 9.6 kg; mean ± SD) and the control group included 16 individuals (age = 34.9 ± 8.0 years, height = 1.62 ± 0.06 m, weight = 61.1 ± 6.3 Kg; mean ± SD). Possible candidates with recent osteoarticular and musculotendinous injury or surgery of lower extremities, background and signs of neurological dysfunction or under medication which could affect motor performance and balance and individuals who had used unstable footwear (specifically Masai Barefoot Technology) prior to the study, were excluded.

The study was conducted according to the involved Institutions' ethical norms and conformed to the Declaration of Helsinki, being informed consent obtained from all participants.

2.2 Instrumentation

The electromyographic (EMG) activity of gastrocnemius medialis (GM), tibialis anterior (TA), rectus femoris (RF) and biceps femoris (BF) muscles was monitored using the *MP 150 Workstation* model from *Biopac Systems*, *Inc. (USA)*, bipolar steel surface electrodes, spaced 20 mm apart, and a ground electrode (*Biopac Systems, Inc.*). The EMG signal was collected at 1000 Hz, pre-amplified at the electrode site and then fed into a differential amplifier with adjustable gain setting (12-500 Hz; Common Mode Rejection Ratio (CMRR): 95 dB at 50 Hz and input impedance of 100 MΩ). The gain range used was 1000. Electrodes were placed at the centre of the muscle belly of GM, TA, RF and BF (Table 1) after the skin was shaved, cleaned with alcohol and scrubbed to reduce impedance to at least 5000 Ω, measured through an Electrode Impedance Checker (Noraxon USA, Inc.). Stabilometry parameters in the horizontal plane (2D) and along the anteroposterior orthogonal axes (Winter, et al., 1998) were obtained using a force plate, model FP4060-10 from *Bertec Corporation (U.S.A)*, connected to a Bertec AM 6300 amplifier, with default gains and a 1000 Hz sampling rate. The amplifier was connected to a Biopac 16 bit analogical-digital converter.

Table 1: Anatomical references to electrode placement. Electrode locations were confirmed by palpation of the muscular belly with the subject in the test position, being the electrodes placed on the most prominent area.

Muscle	Electrode placement
TA	1/3 on the line between the tip of the tibia and the tip of the medial malleolus
GM	Most prominent bulge of the muscle
RF	1/2 on the line from the anterior spina iliaca to the superior border of the patella
BF	1/2 on the line from the ischial tuberosity and the lateral epicondyle of the tibia
RA	3 cm to the right of the umbilicus
ES	2 finger width lateral from the spinous process of L1
Ground electrode	Patella centre

2.3 Procedures

2.3.1 Data collection

In the experimental group, EMG and stabilometric data were acquired in two conditions: (1) prior to using the unstable shoes and (2) after wearing them for a period of 8 weeks. Subjects in the control group were also assessed at two moments separated by 8 weeks, but using a conventional shoe between them. In both groups and in all assessments the variables evaluated were monitored under two randomised conditions: (1) upright barefoot standing and (2) upright standing wearing the unstable shoes (Figure 1). EMG measurements were performed on the dominant limb, determined by asking participants to kick a ball (all participants were right leg dominant). Before data acquisition, all subjects underwent an instruction session by a qualified instructor who explained how to use the unstable shoe, followed by approximately 10 minutes of walking, until the instructor felt they walked properly and were comfortable using the shoes (Nigg, Hintzen, et al., 2006).

Data acquisition was initiated 3 seconds after starting the testing procedure and was done in a total of 3 trials (Pinsault & Vuillerme, 2009; Ruhe, et al., 2010). All individuals were asked to stand as still as possible (Zok et al., 2008), with the support base aligned at shoulder width, keeping their arms by their sides and to focus on a target 2 meters away and at eye level during 30 seconds (Le Clair & Riach, 1996). Rest periods of 60s were provided between trials, during which the subjects sat down while maintaining the foot position (Kitabayashi et al., 2003).

After upright standing measurements and a warm-up consisting of 3 submaximal isometric contractions (Lehman & McGill, 1999), the EMG maximal isometric contraction (MIC) was acquired for signal normalisation. For the TA and GM the ankle was placed in neutral position, and for the BF and RF the knee was at 90º. All participants were asked to perform 3 trials of MIC for dorsiflexion, plantarflexion, knee flexion and knee extension, respectively, under resistance, during 5 seconds, with a 60-second rest between trials (Brown & Weir, 2001). The signals collected within the first and last seconds were discarded.

Following an initial evaluation, subjects in the experimental group were given a pair of the unstable shoes, being instructed to wear them as much as possible at least 8 hours a day, 5 days a week, for 8 weeks, to obtain training effects (Nigg, Hintzen, et al., 2006; Ramstrand, et al., 2008; Ramstrand, et al., 2010; Romkes, et al., 2006), and received a guide on how to use the shoes. Participants in the control group were told to continue their normal activities and not begin in any new exercise regime.

2.3.2 Data processing

a) Electromyography

The raw EMG signal was band-pass filtered (20-450 Hz) and the root mean square (RMS) was calculated. The EMG of each muscle was normalised to the corresponding value obtained during MIC (EGMnorm).

The CCI of agonist (GM, BF) – antagonist (TA, RF) muscles at joint level and that of dorsal (GM+BF) – ventral (TA+RF) at muscle group level were calculated using the equations adapted from (Kellis et al., 2003):

i. CCI at joint level – leg segment

$$CCI = \frac{EMGnorm_{TA}}{EMGnorm_{GM} + EMGnorm_{TA}} \times 100;$$

ii. CCI at joint level – thigh segment

$$CCI = \frac{EMGnorm_{RF}}{EMGnorm_{(GM+BF)} + EMGnorm_{RF}} \times 100;$$

iii. CCI at muscle group level

$$CCI = \frac{EMGnorm_{(TA+RF)}}{EMGnorm_{(GM+BF)} + EMGnorm_{(TA+RF)}} \times 100.$$

The R values of the agonist-antagonist muscles at joint and muscle group levels were calculated as (Slijper & Latash, 2004):

a) R at joint level – leg segment

$$R = EMGnorm_{GM} - EMGnorm_{TA};$$

b) R at joint level – thigh segment

$$R = EMGnorm_{(BF+GM)} - EMGnorm_{RF};$$

c) R at muscle group level

$$R = EMGnorm_{(GM+BF)} - EMGnorm_{(TA+RF)}.$$

ii) Stabilometry

A fourth-order, zero phase-lag, low-pass Butterworth filter with a cut-off frequency of 10 Hz (Ruhe, et al., 2010) was applied to all CoP displacement time series. The peak-to-peak amplitude (*P-P*), the mean velocity (*MV*), which was defined as the total CoP displacement divided by the total period, and the dispersion time series estimated by *RMS* were calculated for the anteroposterior direction. In the 2D domain, a 95% confidence ellipse for each trial was estimated to enclose approximately 95% of the CoP motion points. These parameters were selected as they were demonstrated to be sensitive to postural performance and efficiency (Rocchi, et al., 2004).

The RM and TR displacement components were obtained according to the method proposed in (Zatsiorsky & Duarte, 1999). In brief, the RM was calculated by interpolating the instantaneous equilibrium points, defined as the position of CoP when the resulting horizontal force is equal to zero. The difference between the RM and CoP trajectories was defined as the TR component. From the rambling and trembling time series *RMS*, *area*, *MV* and *P-P* variation in the anteroposterior direction were calculated. Data analysis was performed using the Matlab software (MathWorks, USA).

2.4 Statistics

Statistical analysis was processed using Statistic Package Social Science (SPSS) from IBM Company (USA). The sample was characterised by descriptive statistics. The differences in total agonist and antagonist muscle activity, CCI and R values and stabilometric data between the first and second evaluation and between barefoot and unstable shoe conditions were analysed according to the Friedman test, as the data did not follow a normal distribution. To analyse differences between the two groups, the Mann-Whitney U test was used to compare electromyographic and stabilometric data, as this data did not follow a normal distribution.

3. RESULTS

To investigate the effect of USW on postural control, values of stabilometry and of agonist and antagonist relation in the experimental group were compared to reference values obtained in: 1) the unstable shoe condition of the control group; 2) the barefoot condition of the experimental group; 3) and the first evaluation of the experimental group in the unstable shoe condition.

3.1 Influence of USW on stabilometry

3.1.1 USW led to higher CoP displacement before and after prolonged *exposition*

No differences were observed in CoP related values between the first and second measurements neither between groups in both measurements. Globally, higher values of CoP displacement were found in the unstable shoe condition in relation to the barefoot condition. The parameters showing more differences were the *area* and the *RMS* of CoP displacement (Table 2).

3.1.2 USW led to improved postural control in response to prolonged exposition

As to the relation between shifts of CoP and CoM (Table 2), the results demonstrate that USW led to: 1) decreased *RMS* of the RM component comparing to the first evaluation and to the barefoot condition; 2) increased *MV* of the RM component comparing to the first evaluation, to the barefoot condition and to the control group ($p=0.023$); 3) decreased RM *P-P* comparing to the barefoot condition and to the control group ($p=0.049$); 4) increased TR *P-P* comparing to the first evaluation; and 5) decreased TR *RMS* comparing to control group ($p=0.008$).

3.2 Influence of USW on CCI and R levels at joint and muscle group levels

According to Figure 2 no differences were observed in CCI values between the first and second evaluations, in both groups. Also, no differences were observed between groups in the first and second evaluations. However, participants in the experimental group have shown lower CCI values in the unstable shoe condition in relation to the barefoot condition in the first evaluation, which was not observed after 8 weeks of USW.

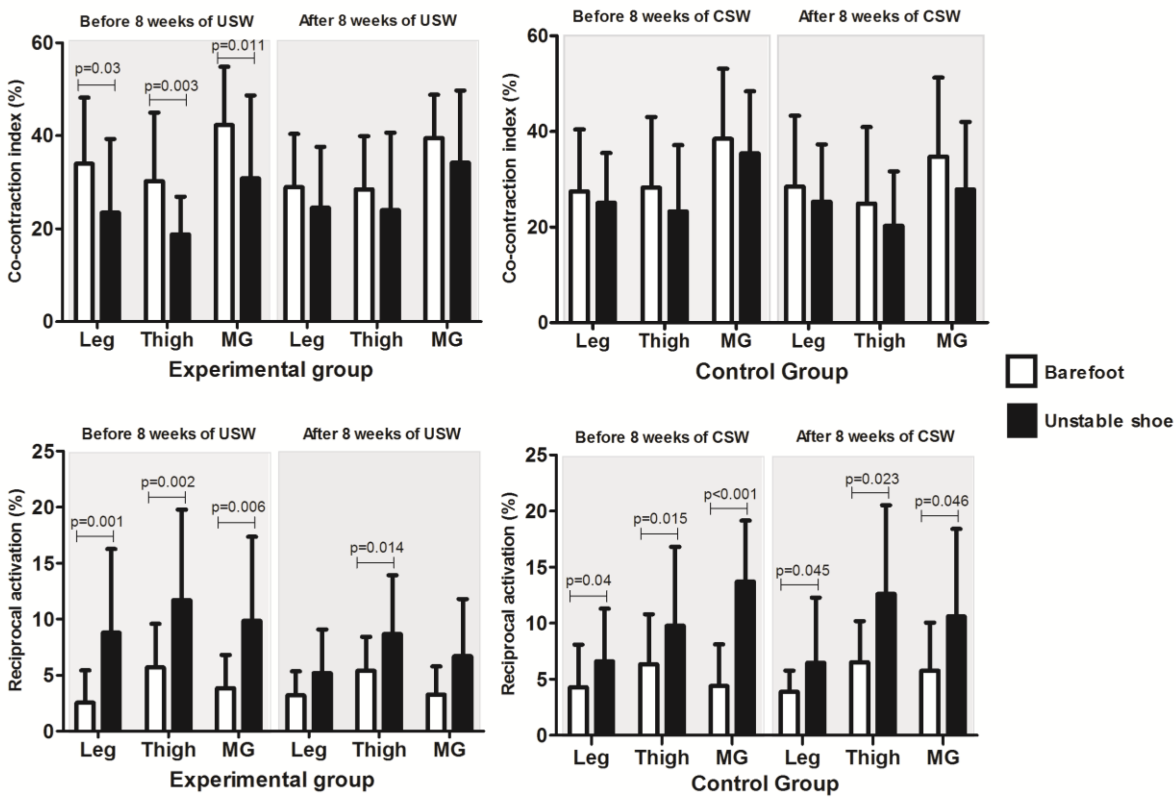

Figure 2: CCI and R values at leg, thigh and muscle group (MG) levels obtained during upright standing before and after 8 weeks of USW in the experimental group and before and after the same period of conventional shoes wearing (CSW) in the control group.

As with CCI values, no differences were observed in R values between the first and second evaluations in both groups. Also, no differences were observed in R values between groups in the first and second evaluations. In the first evaluation, R values were higher in the unstable shoe condition than in the barefoot condition in both groups. In the second evaluation, these differences were maintained in the control group; however, differences were only observed in the experimental group at the thigh level (Figure 2).

There were no differences in total agonist and antagonist activity between the first and second evaluations neither between groups (Figure 3). In all evaluations there was higher agonist muscle activity in the unstable shoe condition than in the barefoot condition.

Table 2: Mean ± standard deviation values of stabilometry parameters obtained in the barefoot and in the unstable shoe conditions before (**1**) and after (**2**) 8 weeks of WUS in the experimental group and before (**1**) and after (**2**) the same period by the control group. Proof values (***p-values***) obtained from comparisons between **1** and **2** in unstable shoe condition and between barefoot and unstable shoe conditions are presented.

Parameters				Experimental group				Control group			
				Barefoot condition	**Unstable shoe condition**	***p-values***		**Barefoot condition**	**Unstable shoe condition**	***p-values***	
Anteroposterior direction	*P-P (mm)*	CoP	**1**	5.75±1.23	5.49±1.16	*ns*	*0.016*	6.24±1.16	6.21±1.25	*ns*	*ns*
			2	5.57±1.23	6.19±1.91		*0.002*	5.67±0.87	5.49±0.80		*ns*
		RM	**1**	5.53±1.16	5.59±1.10	*ns*	*0.016*	5.96±1.05	5.98±0.76	*ns*	*0.006*
			2	5.52±1.17	5.40±1.51		*0.002*	5.52±0.85	5.59±0.76		*0.008*
		TR	**1**	1.71±0.39	1.75±0.34	*0.035*	*0.001*	1.89±0.38	1.73±0.38	*ns*	*ns*
			2	1.69±0.54	2.26±1.50		*ns*	1.71±0.21	1.78±0.28		*ns*
	RMS (mm)	CoP	**1**	2.98±0.18	5.02±0.48	*ns*	*0.001*	3.02±0.31	5.88±0.51	*ns*	*0.001*
			2	3.09±0.77	4.23±0.76		*0.004*	2.75±0.50	4.63±1.00		*0.012*
		RM	**1**	2.84±1.55	4.81±1.72	*0.011*	*0.011*	2.63±0.95	5.56±2.02	*ns*	*0.001*
			2	3.32±1.47	2.85±1.79		*0.019*	2.68±2.32	6.78±0.65		*0.002*
		TR	**1**	0.50±0.17	1.75±1.40	*ns*	*0.001*	0.58±0.28	1.64±1.63	*ns*	*0.001*
			2	1.57±0.80	1.65±1.84		*ns*	0.63±0.32	1.89±1.04		*0.001*
	MV (mm.s^{-1})	CoP	**1**	0.253±0.056	0.236±0.050	*ns*	*0.013*	0.271±0.050	0.268±0.055	*ns*	*ns*
			2	0.160±0.035	0.156±0.034		*ns*	0.247±0.038	0.238±0.038		*ns*
		RM	**1**	0.057±0.012	0.052±0.011	*0.009*	*0.016*	0.063±0.012	0.058±0.013	*ns*	*0.006*
			2	0.056±0.017	0.064±0.014		*0.001*	0.057±0.007	0.053±0.009		*0.008*
		TR	**1**	0.184±0.039	0.183±0.037	*ns*	*0.016*	0.199±0.035	0.192±0.025	*ns*	*ns*
			2	0.184±0.039	0.205±0.070		*ns*	0.184±0.028	0.186±0.025		*ns*
Area (mm^2)		CoP	**1**	125±30.8	361±80.9	*ns*	*0.002*	106±26.5	334±50.3	*ns*	*0.001*
			2	124±50.6	214±54.2		*0.005*	88.7±23.8	254±63.7		*0.027*
		RM	**1**	170±145	466±368	*ns*	*0.002*	142±37.6	562±282	*ns*	*0.001*
			2	210±190	452±279		*0.001*	160±116	363±206		*0.003*
		TR	**1**	9.31±6.31	81.9±57.8	*ns*	*0.001*	13.1±12.0	79.9±76.9	*ns*	*0.001*
			2	22.7±18.9	141±187		*0.004*	15.7±10.2	113±94.9		*0.001*

4. Discussion

The results of this study demonstrate that values of the different CoP parameters are generally higher in the unstable shoe condition than in the barefoot condition, which has also been demonstrated in earlier studies (Landry, et al., 2010; Nigg, Hintzen, et al., 2006). Based on these findings, unstable shoes have been reported as promoters of increased instability (Nigg, Hintzen, et al., 2006). However, the training effects associated with prolonged USW have been questioned as no differences were observed in displacement and *MV* of the CoP before and after a period of USW (Ramstrand, et al., 2010; Turbanski, et al., 2011). Our results demonstrate that prolonged USW does not lead to changes in other CoP parameters as well, like the *area* and *RMS*. Nevertheless, it was interesting to note that *MV* was higher in the unstable shoe condition than in the barefoot condition, in the first evaluation, whereas no differences were observed after USW. Considering that *MV* of CoP displacement has been described as a good index of the amount of activity required to maintain stability (Geurts, et al., 1993) and the important role of the gastrocnemius in postural control (Fitzpatrick, Gorman, et al., 1992; Lakie, et al., 2003; Loram & Lakie, 2002a; Loram, et al., 2005a; Maki & Ostrovski, 1993), it can be hypothesised that *MV* changes could result from an adaptation of ankle plantar flexors' response, as a similar pattern was observed in GM activity following USW (Sousa, Tavares, Macedo, et al., 2012).

Training effects from USW were more evident in RM and TR parameters. The reduction of the *RMS* of RM trajectories reflects a higher effectiveness of the postural control system (Murray et al., 1975; Prieto, et al., 1996; van Wegen et al., 2002) related to supraspinal processes that define an instantaneous point about which the body is stabilised (Zatsiorsky & Duarte, 1999, 2000). This adaptation can be also interpreted as an improvement of the postural control system effectiveness in scanning the limits of postural stability (Riley, et al., 1997).The decreased of RM *P-P* indicates a higher performance of supraspinal mechanisms (Bennell & Goldie, 1994; Kinzey, et al., 1997; Norris, et al., 2005) while increased *MV* of the RM component could be related to a reweighted combination of reciprocal activation and coactivation commands (Drew & Rossignol, 1987; Feldman, 1980a, 1980b; Lacquaniti, 1992; Lacquaniti et al., 1991; Levin et al., 1992). Indeed, a relation between larger coactivation commands and faster movements has been stated (Feldman & Levin, 1995). In spite of the non-significant differences, the results of this study reveal a tendency for higher levels of CCI and lower levels of R after USW. Globally, the findings obtained indicate that USW improves performance and effectiveness of supraspinal mechanisms related to postural control in relation to standard barefoot conditions.

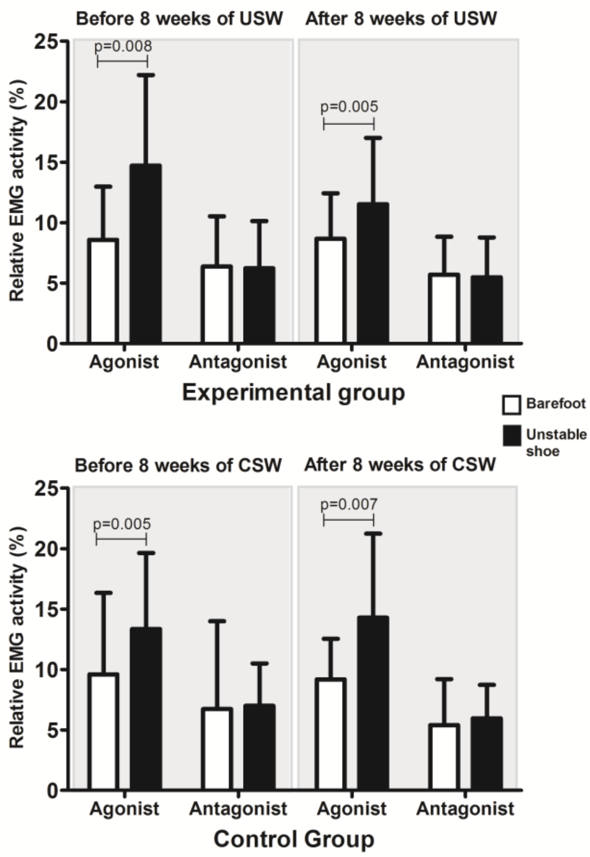

Figure 3: Representation of the mean and standard deviation values of total EMG activity of agonist and antagonist muscles at leg, thigh and muscle group (MG) levels during upright standing in the barefoot and unstable shoe conditions before and after 8 weeks of USW in the experimental group and before and after the same period of conventional shoes wearing (WCS) in the control group.

The reduction of *RMS* of the TR component in the experimental group can be interpreted as a reduction in the "error" signal in the balance control system (Winter, 1995), reflecting increased performance of the action of spinal reflexes and changes in the intrinsic mechanical properties of muscles not only in relation to the first evaluation but also in relation to the barefoot condition. This idea is also supported by the results obtained as to CCI and R values, as there was a tendency for higher values of CCI and lower levels of R after prolonged USW. An interesting finding was obtained from the comparison of TR values between the unstable shoe and barefoot conditions, before and after prolonged USW. In the first evaluation, *RMS* TR values were higher in the unstable shoe condition in relation to the barefoot condition; however, no differences were observed between the two conditions in the second evaluation. Considering that the same changes were observed in

CCI and R, it can be hypothesised that: (1) the higher *RMS* of TR values observed in the unstable shoe condition before prolonged USW can be associated to the lower CCI and higher R levels observed at leg, thigh and muscle group levels; and (2) the non-existence of differences between the two conditions in the second evaluation can be related to the non-existence of differences in CCI and R values between the two conditions. The changes observed in CCI and R values are in accordance with the evidence that training on unstable ground induces a suppression of the H-reflex as a result of modulation of presynaptic inhibition of Ia afferents (Gruber et al., 2007; Taube et al., 2007). The CNS weighs the viscoelastic force (through intrinsic muscle properties and spinal reflex) more strongly at the beginning of learning (Flash, 1987), when the internal models are poor, and it gradually increases the internal model contribution as the learning proceeds (Imamizu et al., 2000; Osu et al., 2002) in order to decrease the motor command amplitude by decreasing the viscoelastic force, reducing the noise and increasing accuracy (Lacquaniti et al., 1993).

In spite of the adaptations mentioned, the total agonist activity and CoP fluctuation in the unstable shoe condition are still higher than in the barefoot condition, suggesting that the destabilising effect of the unstable shoe remains even after the extended use of unstable shoes. Commonly chosen ergonomic intervention methods to reduce pain and discomfort associated with prolonged standing are the alteration of the flooring on which workers stand, and the use of in-soles in the footwear (King, 2002), as one of the strategies is to make the body sway naturally and imperceptibly. Considering this, the results of the present study suggest that USW could be a beneficial ergonomic intervention. However, studies on the influence of USW on subjective rating of fatigue and discomfort while standing are demanded to support our hypothesis.

5. CONCLUSION

The results obtained demonstrate that USW provides increased effectiveness and performance of supraspinal processes and of spinal reflexes and/or in the intrinsic mechanical properties of muscles and joints leading to improved performance of the standing postural control system. The results also demonstrate that the instability provided by USW in standing postural control remains even after adaptation by the postural control system. Although quiet standing research allows studying certain aspects of postural control, it presents some limitations in revealing balance mechanisms and as a diagnostic tool to pinpoint deficits of the system (Winter, 1995). In fact, postural adjustment cannot be deduced from quiet standing posture alone (Moya, et al., 2009). As a consequence, the study of the influence of USW in upright postural control should be implemented in conditions of higher postural challenge.

PART B – *ARTICLE VIII*

The influence of wearing unstable shoes on compensatory control of posture

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ABSTRACT

Purpose: This study investigated the influence of wearing unstable shoes (WUS) on compensatory postural adjustments (CPA) associated to an external perturbation. *Methods:* Subjects (n=32) stood on a force platform resisting an anterior-posterior horizontal force applied to a pelvic belt via a cable, which was suddenly released, under two conditions: barefoot and WUS. The electromyographic activity (EMGa) of *gastrocnemius medialis* (GM), *tibialis anterior* (TA), *rectus femoris* (RF), *biceps femoris* (BF), *rectus abdominis* (RA), and *erector spinae* (ES) muscles and the centre of pressure (CoP) displacement were acquired to study CPA. EMGa was used to assess individual muscle activity and latency, co-contraction index (CCI) and reciprocal activation (R) at joint and muscle group levels. *Results:* Compared to barefoot, WUS led to: (1) increased EMGa of GM, (2) increased total agonist activity, (3) decreased CCI at the ankle joint and muscle group levels, (4) an increase of R at the ankle joint and muscle group levels, and (5) a decrease of all muscle latencies. No differences were observed in CoP displacement between conditions. Conclusion*:* WUS leads to changes in neuro-muscular mechanisms that are responsible for the control and monitoring of postural sway during both standing and in response to an external perturbation without compromising the performance of postural control.

***Key words*:** Posture; External perturbation; Compensatory Postural Adjustments; Electromyography; Centre of pressure.

1. INTRODUCTION

The ability to compensate for external perturbations is important to prevent falls and to ensure safe and independent mobility (Mochizuki et al., 2009). Evoked compensatory postural muscle responses are produced when instability occurs (Britton et al., 1993) and are of shorter latency than voluntary activation of the same muscles (Gage et al., 2007; Maki & McIlroy, 1997). Despite a very rapid latency, the balance-recovery reactions are remarkably complex. Triggered and modulated by multiple sensory inputs, these reactions are highly adaptable to meet functional demands, as defined by the features of the perturbation, the ‘‘central set’’ of the individual, ongoing cognitive or motor activity, environmental constraints on reaction-force generation and limb movement and the postural configuration adopted by the subject (Forssberg & Hirschfeld, 1994; Henry et al., 2001; Horak et al., 1989; Maki & McIlroy, 2007).

It is well known that human standing is successfully maintained using visual, vestibular and somatosensory information. Proprioceptive information originating from sensory receptors in the lower limb (Horak & Nashner, 1986; Inglis et al., 1994) or in the more proximal joints (Bloem et al., 2002) has been identified as a key source of triggering information needed to initiate directionally specific, automatic postural responses following an unexpected postural perturbation. It is known that during quiet standing, sway of the entire body is highly correlated with ankle joint rotation, which shows that muscles crossing the ankle joint are able to provide the sensory information necessary to maintain upright standing (Fitzpatrick, et al., 1994; Fitzpatrick, Gorman, et al., 1992; Gatev, et al., 1999; Loram, et al., 2005a; Nashner, 1982). The ankle joint muscle proprioceptors which might provide this sensory information include those in calf muscles and the tibialis anterior. Ankle plantar flexors act as active agonists and, because the foot is constrained on the ground, these muscles prevent forward toppling of the body whose centre of gravity is maintained in front of the ankle joint (Fitzpatrick, Gorman, et al., 1992; Lakie, et al., 2003; Loram & Lakie, 2002a; Loram, et al., 2005a; Maki & Ostrovski, 1993). The main antagonist, tibialis anterior, may be a source of muscle proprioceptive input when stretched by body sway. However, these roles are dynamic, and apparently reversible according to the position of the centre of mass in relation to the ankle joint (Di Giulio, et al., 2009).

Recently, shoe manufacturers have introduced specific shoes featuring unstable sole constructions to induce neuromuscular stimuli similar to balance training, e.g. Masai Barefoot Technology (MBT) shoes. MBT shoes are characterised by a rounded sole in the anterior–posterior direction with a soft pad underneath the rear foot and are supposed to increase microscopic movement variability during standing (Nigg, Hintzen, et al., 2006) and walking (Stöggl et al., 2010), thus enhancing sensory feedback to the locomotor system (Collins et al., 2003). It has been demonstrated that wearing this kind of unstable shoe leads to changes in the ankle control pattern during quiet standing (Landry, et al., 2010; Sousa, Tavares, Rodrigues, et al., 2012) and gait (Romkes, et al., 2006). However, most studies related to postural control have been focused on centre of pressure excursions (Landry, et al., 2010; Ramstrand, et al., 2010; Turbanski, et al., 2011). To the best of our knowledge, no previous study has analysed the influence of unstable shoe wearing on muscle compensatory responses.

The main purpose of this study was to analyse the influence of wearing unstable shoes (WUS) on compensatory postural adjustments (CPA) to an external perturbation.

More specifically, the purposes were to evaluate the effect of WUS on: 1) muscle latency and activity and 2) centre of pressure (CoP) displacement associated to an external perturbation. Since postural responses involve activation of muscle synergies throughout the entire body and are also more context-specific, more flexible and adaptable than spinal proprioceptive reflexes (Horak & Macpherson, 1996b), muscle activity was analysed not only in terms of individual magnitude but also in terms of degree of co-contraction index (CCI) and reciprocal activation (R) at joint and muscle group levels. The results of this study contribute to understand how WUS affects postural control.

2. METHODOLOGY

2.1 Subjects

Thirty-two healthy female subjects (age = 34 ± 9 years, height = 1.61 ± 0.06 m, weight = 63.2 ± 9.3 kg; mean ± SD) took part in the experiment; possible candidates were excluded if they presented a recent osteoarticular and musculotendinous injury or surgery of lower extremities, a background of or signs of neurological dysfunction or medication which could affect motor performance and balance and individuals who had used unstable footwear (specifically Masai Barefoot Technology - MBT, Figure 1) prior to the study.

Figure 1: Unstable shoe model used in this study: The Masai Barefoot Technology (MBT) shoe has a rounded sole in the anterior-posterior direction, thus providing an unstable base

The study was conducted according to the ethical norms of the Institutions involved and conformed to the Declaration of Helsinki, with informed consent from all participants.

2.2 Instrumentation

The electromyographic activity (EMGa) of gastrocnemius medialis (GM), tibialis anterior (TA), rectus femoris (RF), biceps femoris (BF), rectus abdominis (RA), and erector spinae (ES) muscles were monitored using the MP 150 Workstation model from

Biopac Systems, Inc. (USA), with steel surface electrodes, TD150 model, bipolar configuration, with a 20 mm interelectrode distance and a ground electrode. The selection of these muscles was based on the fact that the unstable shoes used in this study have a rounded sole in the anterior-posterior (AP) direction and the perturbation was applied in the same direction.

CoP values were obtained using a force plate, model FP4060-10 from Bertec Corporation (USA), connected to a Bertec AM 6300 amplifier, with default gains and a 1000 Hz sampling rate. The amplifier was connected to a Biopac 16 bit analog-to-digital converter.

2.3 Procedures

2.3.1 Skin preparation and electrode placement

The subjects' lower limb skin surfaces were prepared to reduce electrical resistance to less than 5000 Ω. Measurement electrodes were placed at GM, TA, RF, BF, ES and RA mid-belly according to anatomical references (Table 1) and fixed with adhesive tape (Basmajian & De Luca, 1985; Hermens, et al., 2000).

Table 1: Anatomical references used to locate the electrodes. Electrode locations were confirmed by palpation of the muscular belly with the subject in the test position; the electrodes were placed on the most prominent area.

Muscle	Electrode placement
TA	A third way along the line between the tip of the tibia and the tip of the medial malleolus
GM	Most prominent bulge of the muscle
RF	Halfway along the line from the anterior spina iliaca to the superior border of the patella
BF	Halfway along the line from the ischial tuberosity and the lateral epicondyle of the tibia
RA	Three cm to the right of the umbilicus
ES	Two finger widths lateral from the spinous process of L1
Ground electrode	Patella centre

2.3.2 Experimental setup

Each subject performed two tests: one standing barefoot and another WUS. Subjects were instructed to stand relaxed, with feet comfortably spaced and arms at sides, and to look straight ahead to a target set 2 m away (Fransson et al., 1999). Headphones were used to listen to music to mask any auditory cues and to distract the subject from consciously modifying her motion. A horizontal cable was attached to a pelvic belt worn by the subjects while they kept their bodies essentially straight. A backward force of 5% of body weight (Krebs et al., 2001; Wolfson, et al., 1986), measured with an isometric dynamometer, was applied to the cable for a random period of 3 to 10 seconds and then the cable was released (time zero, T_0). A 1-minute rest interval was set between each test to prevent fatigue (Maki, 1986). Test instructions to the subject were: "Stand still but compensate the force applied to the belt without moving your feet. I will let go at some point, but you will not know when. Do not move your feet, but keep your balance." The results obtained in a pilot study as to the inclination of the unstable shoe after applying the horizontal force demonstrated that the ankle dorsiflexion angle was not greater than 5º, which is not enough to produce changes in group Ia afferent feedback or in plantar and dorsiflexor muscle activity levels (Mezzarane & Kohn, 2007). Each subject performed two randomised series, one for each condition under study, of three trials each. As no noteworthy differences were verified between the first and the remainder of the trials, the average values were used for analysis. Measurements were performed on the dominant limb, which was the right limb. Before data acquisition, all subjects were given time to become familiar with the test environment (Maki, 1986) and were explained by a qualified instructor on how to use the unstable shoe, followed by approximately 10 minutes of walking, until the instructor felt they walked properly and were comfortable using the shoes (Nigg, Hintzen, et al., 2006).

The EMG signals were acquired according to a sample rate of 1000 Hz, pre-amplified at the electrode site, fed into a differential amplifier with an adjustable gain setting (12-500 Hz; Common Mode Rejection Ratio (CMRR): 95 dB at 60 Hz and input impedance of 100 MΩ), digitised and then stored in a computer for subsequent analysis based on the Acqknowledge software (Biopac Systems, Inc. USA). The gain range was set to 1000.

The muscle latency was detected in a time window from -450 to +200 ms in relation to T_0 (Santos, et al., 2009) using a combination of computational algorithms and visual inspection to ensure against false-positive identifications of onset times, as recommended in (Di Fabio, 1987). The latency for a specific muscle was defined as the instant lasting for at least 50 ms (Hodges & Bui, 1996) when its EMG amplitude was greater (activation) or smaller (inhibition) than the mean of its baseline value plus 1 (one) standard deviation (SD) (Hodges & Bui, 1996), measured from -500 to -450 ms (Santos, et al., 2009). The signal was previously smoothed using a sixth order elliptical low-pass software filter of 50 Hz, based on the findings described in (Hodges & Bui, 1996).

The GM activity of TA, GM, RF, BF, ES and RA was evaluated at pre-defined epochs. To assess the level of muscle activity, signals were previously band-pass filtered between 20 and 450 Hz and integrated with 150 ms time windows. The integral of EMG activity (Int_{EMGi}) was analysed at two epochs in relation to T_0: 1) 50 to 200 ms (compensatory postural adjustments 1 (CPA1)), and 2) 200 to 350 ms (late compensatory postural adjustments (CPA2)) (Latash, 2008; Santos, et al., 2009). The Int_{EMGi} for each epoch was corrected by the triple of integral of basal EMG activity from -500 to -450 in relation to T_0 (Aruin & Latash, 1995b; Santos, et al., 2009). It should be noted that positive values indicate increased muscle activation, while negative values indicate a decrease in relation to background activity. Then, the Int_{EMGi} data were normalised to maximal isometric force for each subject (EMG_{norm}). After a warm-up consisting of 3 submaximal isometric contractions (Lehman & McGill, 1999) the TA and MG maximal isometric contractions were measured with the ankle in a neutral position, for the BF and RF the knee was positioned at 90º and for ES and RA subjects were lying in prone and supine position, respectively. Manual resistance was applied to all muscles.

The CCI of EMG activity of antagonist (TA, RF, RA) – agonist (GM, BF, ES) muscle pairs at joint level (leg and thigh segments) and of ventral (TA + RF + RA) – dorsal (GM + BF + ES) muscles at a muscle group level were calculated using equations adapted from (Kellis, et al., 2003):

1. Joint level – leg segment:

$$CCI = \frac{EMGnorm_{TA}}{EMGnorm_{GM}+EMGnorm_{TA}} \times 100, \qquad (2)$$

2. Joint level – thigh segment:

$$CCI = \frac{EMGnorm_{BF}}{EMGnorm_{(GM+BF)}+EMGnorm_{BF}} \times 100, \qquad (3)$$

3. Joint level – trunk segment:

$$CCI = \frac{EMGnorm_{RA}}{EMGnorm_{ES}+EMGnorm_{RA}} \times 100, \qquad (4)$$

4. Muscle group level

$$CCI = \frac{EMGnorm_{(TA+RF+RA)}}{EMGnorm_{(GM+BF+ES)} + EMGnorm_{(TA+RF+RA)}} \times 100. \quad (5)$$

This method provides an estimate of the relative activation of the pair of muscles as well as the co-contraction magnitude.

The R of antagonist-agonist muscles at the joint level and ventral-dorsal muscles at the muscle group level were calculated using the following equations, proposed by (Slijper & Latash, 2004):

1. Joint level - leg segment:

$$R = EMGnorm_{GM} - EMGnorm_{TA}, \quad (6)$$

2. Joint level – thigh segment:

$$R = EMGnorm_{(GM+BF)} - EMGnorm_{RF}, \quad (7)$$

3. Joint level – trunk segment:

$$R = EMGnorm_{ES} - EMGnorm_{RA}, \quad (8)$$

4. Muscle group level:

$$R = EMGnorm_{(GM+BF+ES)} - EMGnorm_{(TA+RF+RA)}. \quad (9)$$

The acquired force time series of each trial were used to calculate the CoP fluctuation in the AP direction (as the perturbations were induced symmetrically) using the approximation (Winter, et al., 1998):

$$COP_{AP} = \frac{M_x}{F_z}, \quad (10)$$

where M_x is the moment in the sagittal plane and F_z is the vertical component of the ground reaction force. A fourth-order, zero phase-lag, low-pass Butterworth filter with a cut-off frequency of 20 Hz (Prieto, et al., 1996; Vette et al., 2007) was applied to all CoP displacement time series. The AP SD (SD_{AP}) and peak-to-peak ($P\text{-}P_{AP}$) distance of the CoP were measured in the following epochs: (1) +100 to +250 ms (CPA1); (2) +250 to +400 ms (CPA2). These values were selected to compensate for the electromechanical delay and were corrected as to basal values (obtained during unperturbed standing) (Cavanagh & Komi, 1979; Howatson et al., 2009).

2.4 Statistics

The data were analysed using the software Statistic Package Social Science (SPSS) from IBM Company (USA). Differences between WUS and barefoot conditions and between CPA1 and CPA2 in terms of individual muscle activation, CCI and R at joint and muscle group levels and CoP displacement were analysed using the Friedman ANOVA test. Muscle onset and offset between the two conditions were evaluated using the Wilcoxon test.

3. RESULTS

3.1 Influence on EMG activity in CPA at individual, joint and muscle group levels

WUS led to an increased EMG activity of GM and total agonist activity compared to barefoot (Table 2). The GM activity was higher in CPA1 than in CPA2 for both conditions, while the opposite was verified in TA (Table 3).

Measurements obtained when WUS gave a lower value of CCI at the leg (CPA1, $p<0.0001$; CPA2, $p<0.0001$) and a lower value at the thigh in CPA2 ($p=0.013$), Figure 2. In both conditions CCI was higher in CPA2 at thigh and in barefoot was also higher at leg. At the muscle group level (Figure 2) the CCI was also lower in CPA2 ($p=0.006$) when WUS. Additionally, the CCI at the muscle group level was higher in CPA2 than in CPA1 for both conditions (Table 3).

Table 2: Mean and standard deviation values for GM, TA, BF, RF, ES and RA EMG activity and total agonist and antagonist muscle activity obtained during CPA1 and CPA2 associated to an external perturbation, with and without unstable footwear. Only significant values are expressed numerically non-significant values are represented as ns.

	Muscle	Condition	Mean (%)	Standard Deviation	p-value
CPA1	GM	Barefoot	3.86	2.22	<0.0001
		Unstable shoe	6.33	3.00	
	TA	Barefoot	-0.35	0.77	ns
		Unstable shoe	-0.59	1.25	
	BF	Barefoot	0.70	0.59	ns
		Unstable shoe	0.66	0.52	
	RF	Barefoot	0.29	0.17	ns
		Unstable shoe	0.37	0.26	
	ES	Barefoot	0.62	0.68	ns
		Unstable shoe	0.54	0.36	
	RA	Barefoot	0.36	0.24	ns
		Unstable shoe	0.38	0.28	
	Total agonist	Barefoot	5.17	2.48	P<0.0001
		Unstable shoe	7.52	3.19	
	Total antagonist	Barefoot	0.3	0.87	ns
		Unstable shoe	0.16	1.20	
	CoP	**Condition**	**Mean (m)**	**Standard Deviation**	**p-value**
	$P\text{-}P_{AP}$	Barefoot	0.0395	0.0132	ns
		Unstable shoe	0.0421	0.0132	
	SD_{AP}	Barefoot	0.0118	0.0041	ns
		Unstable shoe	0.0125	0.0042	
	Muscle	**Condition**	**Mean (m)**	**Standard Deviation**	**p-value**
CPA2	GM	Barefoot	2.35	1.79	<0.0001
		Unstable shoe	4.56	2.42	
	TA	Barefoot	-0.49	0.89	ns
		Unstable shoe	-0.77	1.36	
	BF	Barefoot	0.58	0.54	ns
		Unstable shoe	0.62	0.52	
	RF	Barefoot	0.28	0.21	ns
		Unstable shoe	0.34	0.23	
	ES	Barefoot	0.54	0.54	ns
		Unstable shoe	0.55	0.32	
	RA	Barefoot	0.33	0.21	ns
		Unstable shoe	0.36	0.26	
	Total agonist	Barefoot	3.47	1.89	P<0.0001
		Unstable shoe	5.73	2.55	
	Total antagonist	Barefoot	0.12	1.02	ns
		Unstable shoe	0.08	1.35	
	CoP	**Condition**	**Mean (m)**	**Standard Deviation**	**p-value**
	$P\text{-}P_{AP}$	Barefoot	0.0039	0.0014	ns
		Unstable shoe	0.0043	0.0025	
	SD_{AP}	Barefoot	0.0010	0.0004	ns
		Unstable shoe	0.0011	0.0008	

Table 3: Proof values (p-values) obtained from comparisons of individual muscle activity, R and CCI at joint and muscle group level, total agonist and antagonist activity and cop displacement between CPA1 and CPA2. Only significant values are expressed numerically non-significant values are represented as ns.

Level	Variable compared	p-value (CPA1 and CPA2 comparisons)
Individual	TA	Barefoot: $p=0.036$
		Unstable shoe: $p=0.012$
	GM	Barefoot: $p<0.0001$
		Unstable shoe: $p<0.0001$
	RF	Barefoot: ns
		Unstable shoe: ns
	BF	Barefoot: ns
		Unstable shoe: ns
	RA	Barefoot: ns
		Unstable shoe: ns
	ES	Barefoot: ns
		Unstable shoe: ns
Joint	R leg	Barefoot: $p<0.0001$
		Unstable shoe: $p<0.0001$
	R thigh	Barefoot: ns
		Unstable shoe: ns
	R trunk	Barefoot: ns
		Unstable shoe: ns
	CCI leg	Barefoot: $p=0.006$
		Unstable shoe: ns
	CCI thigh	Barefoot: $p<0.0001$
		Unstable shoe: $p=0.021$
	CCI trunk	Barefoot: ns
		Unstable shoe: ns
Muscle group	Total agonist	Barefoot: ns
		Unstable shoe: ns
	Total antagonist	Barefoot: ns
		Unstable shoe: ns
	R	Barefoot: $p<0.0001$
		Unstable shoe: $p<0.0001$
	CCI	Barefoot: $p<0.0001$
		Unstable shoe: $p<0.0001$
CoP	$P\text{-}P_{AP}$	Barefoot: ns
		Unstable shoe: ns
	SD_{AP}	Barefoot: ns
		Unstable shoe: ns

Considering R values, Figure 3, WUS condition was associated to a value higher than the one obtained in barefoot for the leg segment (CPA1, $p<0.0001$; CPA2, $p<0.0001$). At this segment, R was higher in CPA2 when WUS and in CPA1 for the barefoot condition. R levels were also higher when WUS at muscle group level (CPA1, $p=0.001$; CPA2, $p=0.001$). In this level there was higher R value in CPA1 than in CPA2 in both conditions.

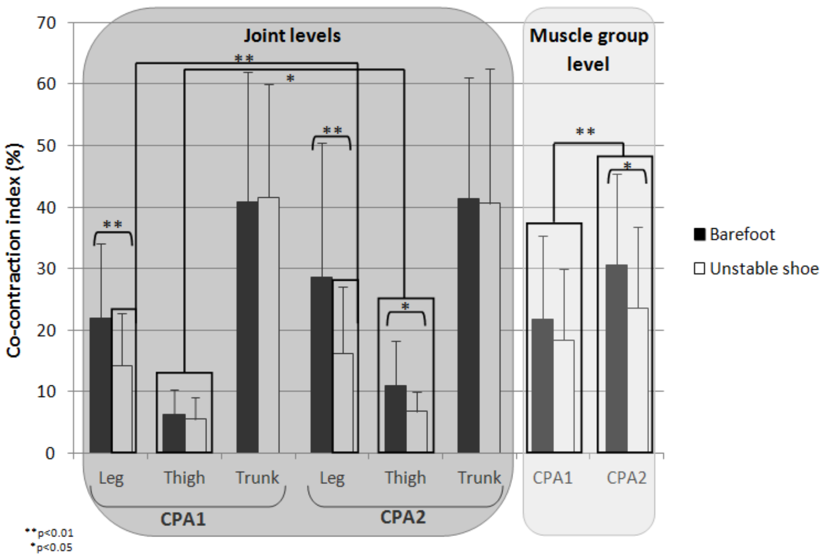


Figure 2: Representation of mean and standard deviation values of co-contraction index at the joint and muscle group levels during CPA in barefoot and wearing unstable shoe.

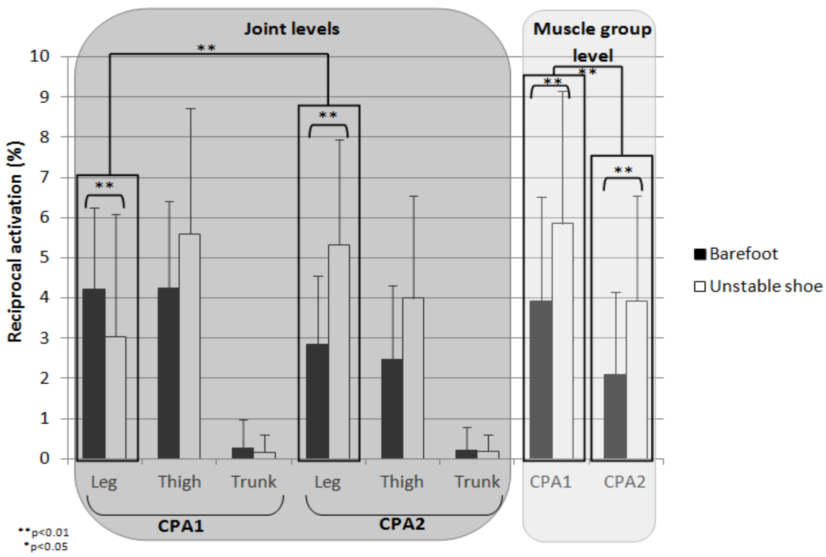
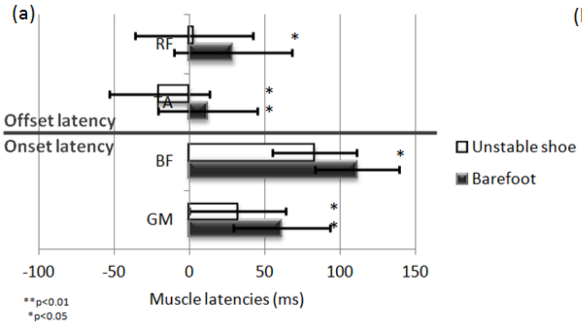


Figure 3: Representation of mean and standard deviation values of reciprocal activation at the joint and muscle group levels during CPA in barefoot and wearing unstable shoe.

3.2 Influence on muscle latency

Average values indicate a distal to proximal activation sequence and a distal to proximal deactivation pattern in barefoot and in WUS. Significant differences occurred between barefoot and when WUS in onset latency of GM and BF ($p=0.001$ and $p=0.016$, respectively) and in offset latency of TA and RF ($p<0.0001$ and $p=0.022$, respectively). In spite of these differences, there were no noteworthy differences in the time between ventral muscle deactivation and dorsal muscle activation in barefoot and in WUS (Figure 4).



(b)

Condition	Muscle deactivation/activation sequence	Occurrences
Barefoot	TA → RF deactivation / GM → BF activation	75%
	RF → TA deactivation / GM → BF activation	12.5%
	TA → RF deactivation / BF → GM activation	9.38%
	RF → TA deactivation / BF → GM activation	3.12%
Unstable shoe	TA → RF deactivation / GM → BF activation	59.38%
	RF → TA deactivation / GM → BF activation	21.88%
	TA → RF deactivation / BF → GM activation	15.62%
	RF → TA deactivation / BF → GM activation	3.12%

Figure 4: (a) Representation of the organisation of the average postural responses (TA and RF offset, GM and BF onset) activated in response to an external perturbation in barefoot and wearing unstable shoe. Muscle latency of trunk muscles were not analysed as no differences were observed at all levels between the two conditions under comparison. (b) Descriptive analysis on muscle deactivation/activation sequences.

3.3 Influence on CoP displacement in CPA

It were observed no statistical significant differences in P-P_{AP} and SD_{AP} between measurements obtained with and without the unstable shoe (Table 2).

4. DISCUSSION

This study aimed to analyse the influence of WUS on CPA at individual muscle, joint and muscle group levels. Starting with the individual muscle level, the results obtained show increased GM activity when WUS, reflecting ankle strategy use. In fact, the major differences in terms of individual muscle activity occurred at the ankle joint, which is in line with other studies that analised quiet standing (Sousa, Tavares, Rodrigues, et al., 2012), gait (Romkes, et al., 2006) and running (Boyer & Andriacchi, 2009). According to Ivanenko, Levik et al. (1997), when standing on a rocking support, usually humans do not move the CoM, shifting instead the point of contact of the rocking platform with the ground under the CoM, which leads to an increased need of gastrocnemius activation (Ivanenko, et al., 1997). Increased GM activity is consistent with values obtained at the muscle group level.

Considering the hypothesis that the activity of all muscles within the system is interdependent (Feldman & Levin, 1995), values of CCI and R were calculated at different joint and at muscle group levels. The decreased antagonist CCI was a surprising finding since "Freezing degrees of freedom" has been described as a primitive strategy when mastering a new skill (Baratta et al., 1988; Bernstein, 1967; De Luca & Mambrito, 1987; Psek & Cafarelli, 1993) and because it has been shown that subjects use co-contraction control to offset the effects of destabilising forces (Burdet et al., 2001; Darainy et al., 2004; Franklin et al., 2003). The higher activity of GM when WUS could be associated to the lower levels for leg antagonist CCI as there is evidence that the amount of inhibition of the antagonists increases in proportion to the level of motor activity in the agonists (Lavoie, et al., 1997). Evidence suggests that the regulation of CCI during a muscle action is continuously controlled by the nervous system (Nielsen & Kagamihara, 1992, 1993), and that it may be centrally mediated by a descending "common drive" (De Luca & Mambrito, 1987). An interesting finding of this study is that in the barefoot condition there was higher leg and muscle group CCI levels in CPA2, which is consistent with the role of the CNS in controlling co-activation. It were observed no differences between CPA1 and CPA2 when WUS. This can be explained by the enhanced reflex excitability that increases the role of the stretch reflex in posture control during standing on an unstable support area (Dietz, et al., 1980). In fact, changes in strategy for maintaining the upright posture to adapt postural control to an unstable support area have been demonstrated (Horak & Nashner, 1986). More specifically, it was shown that the CCI of muscles declines in unstable dynamical tasks (Milner & Cloutier, 1993). The decreased CCI observed when WUS was associated to higher R values for leg and muscle group levels which is consistent with the idea that reciprocal inhibition is stronger in tasks involving joint movement than during voluntary activity of postural maintenance (Lavoie, et al., 1997). The ankle angle in the sagital plane was not measured in this work and thus it would be interesting to explore the effect of this variable in future studies. Another explanation for the higher level of R and lower level of CCI for the ankle joint and muscle group levels when WUS could lie in the muscles analysed. The gastrocnemius is a phasic muscle (Di Giulio, et al., 2009) and as a result it developed higher activity to compensate for the external perturbation. However, in this work, data from the soleus muscle were not acquired and as such the CCI between soleus and TA can be different from that observed between GM and TA, as the soleus is a tonic muscle (Di Giulio, et al., 2009). Although being a phasic muscle, increased activity of the GM muscle has been demonstrated during a co-contraction task, which may indicate that the central command eliciting co-contraction across the ankle joint involves selective activation of this muscle (Nielsen et al., 1994).

Despite a predominance of a distal to proximal deactivation/activation, the results of this study reveal different muscle combinations of temporal organisation that can be explained by the redundancy in the number and function of muscles, as repetitions of the same movement may result from the activation of muscles in distinct combinations (Feldman et al., 1998). However, it is interesting to note that when WUS there was a lower percentage of occurrences of the main pattern (59.38%) in relation to the barefoot condition (75%). Despite decreased short-term response and a decrease of offset latency of TA and RF when WUS, the time between ventral muscles deactivation and dorsal muscles activation in barefoot and when WUS was not different. Thus, it is reasonable to suggest that in some way, when WUS, subjects anticipated the external perturbation, as muscle activation in postural responses to balance perturbations is modulated by sensory feedback due to the perturbation (Kuo, 1995, 2005; Park, et al., 2004; Peterka, 2002) and the initial conditions (Horak & Macpherson, 1996b). Although the specific excitatory and inhibitory inputs cannot be deduced from this study, several possibilities exist. The most probable input may originate from within the muscles, where the small magnitudes of sway observed during quiet standing may be enough to alter muscle lengths. Consequently, these length changes to the distal muscles are likely to result in changes of Ia-afferent input onto the motoneuron pool of the lower limbs. Recent studies by (Loram, et al., 2005b) have suggested this possibility, whereby muscle length changes in the gastrocnemius and soleus muscles during quiet standing have been detected within the range at which muscle spindles are sensitive to movement (Proske, et al., 2000). Taking this into account it would be relevant in future studies to evaluate muscle length changes in triceps surae and TA muscles when WUS. A second possible source of sensory information may come from the cutaneous afferents of the feet as there is a large distribution of cutaneous receptors at various locations on the sole of the foot (Kennedy & Inglis, 2002). It has been suggested that this source of proprioceptive information contributes to both the coding and spatial representation of body posture during standing (Roll, et al., 2002) and that the architecture and physiology of the foot appear to contribute to the task of bipedal postural control with great sensitivity (Wright, et al., 2012). Future studies should analyse the influence of WUS on foot anatomy and sensorimotor control of posture because changes in plantar pressure distribution were found in this condition and values of the average contact area when WUS (Stewart, et al., 2007) are lower than those obtained when standing barefoot (Nicolopoulos et al., 2000). A third possibility, although somewhat debated, is the potential role of the vestibular system during quiet standing and in response to a translation of the support surface. While this sensory system may not be a large contributor for the control of upright stance (Winter et al. 1998), it is likely to play a crucial role during moments of increased postural instability (Fitzpatrick and McCloskey 1994). However, the results obtained by Horak et al., 1990, demonstrate that vestibular inputs are not required for triggering or coordinating the muscle activation patterns associated to ankle strategy. The AP CoP displacement was not altered when WUS suggesting that the performance of the neuromuscular system is not perturbed when subjects wear the unstable shoe. It is important to note that only one kind of unstable shoe (MBT) was tested and consequently, the results of this study should not be generalized to other types of unstable footwear.

5. CONCLUSIONS

The results reveal that WUS led to higher levels of GM activity and total agonist activity, lower levels of co-contraction at leg and thigh, higher levels of reciprocal activation values at leg and early offset/onset latencies at leg and thigh in relation to barefoot measurements. The results also demonstrate that despite these changes the performance of postural control is not perturbed when subjects wear the unstable shoe.

PART B – *ARTICLE IX*

Influence of long-term wearing of unstable shoes on compensatory control of posture: An electromyography-based analysis

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ABSTRACT

Purpose: This study investigated the influence of long-term wearing of unstable shoes (WUS) on electromyographic activity (EMGa) during compensatory postural adjustments (CPA) to an external perturbation. *Methods:* Participants were divided into two groups: one wore unstable shoes while the other wore conventional shoes for 8 weeks. The integral of the EMGa of gastrocnemius medialis (GM), tibialis anterior (TA), rectus femoris (RF) and biceps femoris (BF) muscles, and centre of pressure (CoP) displacement were studied within time intervals typical for CPA under two conditions (barefoot and using the unstable shoe). EMGa was used to assess individual muscle activity and latency, co-contraction index (CCI) and reciprocal activation (R) at the joint and muscle group levels. Results: Long-term WUS led to: an increase of BF activity in both conditions (barefoot and unstable shoe); a decrease of GM activity; an increase of CCI and a decrease of R at leg and muscle group levels in the unstable shoe condition. Additionally, WUS led to a decrease in CoP displacement, however no differences were observed in muscle onset and offset. *Conclusion:* Results suggest that the prolonged use of unstable shoes leads to higher levels of pre-synaptic inhibition at leg and muscle group levels associated to a decreased CoP displacement.

Keywords: Postural control; unstable shoe wearing; adaptation; electromyography.

1. INTRODUCTION

Automatic postural responses to external perturbations are shaped by the sensory characteristics of the perturbation and by central nervous system (CNS) mechanisms related to expectations, attention, experience, environmental context, and intention, as well as by pre-programmed muscle activation patterns called synergies (Horak, 1996). Studies concerning postural perturbations have shown that postural response strategies become more efficient and effective in response to repeated exposure to a destabilising stimulus, as the automatic postural responses are gradually reduced in magnitude, and fewer or different muscles are recruited (Akram, et al., 2008).

The underlying neural adaptations to balance training were shown to occur at different sites of the CNS. Recent studies have demonstrated that training on unstable ground induces a decrease of corticospinal excitability and a suppression of the H-reflex as a result of modulation of presynaptic inhibition of Ia afferents (Gruber, et al., 2007; Taube, et al., 2007). Exercises are commonly performed on ankle disks, balance boards, soft mats and unstable surfaces like 'wobble boards'. Recently, manufacturers have introduced specific shoes featuring unstable sole constructions to induce similar neuromuscular training stimuli. Previous research reported that these shoes improved reactive balance in children with development disabilities (Ramstrand, et al., 2008), improved static and dynamic balance in adults with osteoarthritis (Nigg, Emery, et al., 2006) and in mid age adults (Landry, et al., 2010; Ramstrand, et al., 2010), and also in young subjects in dynamic conditions like standing on a moveable platform (Turbanski, et al., 2011). Electromyography studies reveal changes in the ankle joint during quiet standing (Landry, et al., 2010; Sousa, Tavares, Rodrigues, et al., 2012) and during gait and running (Boyer & Andriacchi, 2009; Romkes, et al., 2006). These are important findings since standing sway is highly correlated with ankle joint rotation, as muscles crossing this joint are able to provide the sensory information required to maintain upright standing (Fitzpatrick, et al., 1994; Loram, et al., 2005b).

The main purpose of this study was to analyse the influence of long-term wearing of unstable shoes (WUS) on compensatory postural adjustments (CPA) to an external perturbation in terms of muscle latency and activity and centre of pressure (CoP) displacement. Because postural responses involve activation of muscle synergies throughout the entire body and are also more context-specific, flexible and adaptable than spinal proprioceptive reflexes (Horak & Macpherson, 1996b), muscle activity was analysed not only in terms of individual magnitude but also in terms of degree of co-contraction index (CCI) and reciprocal activation (R) at joint and muscle group levels (Slijper & Latash, 2004). Based on this, we hypothesised a reduction in muscle activity and latency, changes in CCI and R values, and a reduction in CoP displacement for long-term WUS. To the best of our knowledge, no previous study has analysed the influence of WUS on these variables.

2. METHODS

2.1 Subjects

The study included 30 healthy female individuals distributed in two groups. The experimental group (EG) included 14 individuals (age = 34.6 ± 7.7 years, height = 1.59 ± 0.06 m, weight = 65.3 ± 9.6 kg; mean ± SD) and the control group (CG) included 16 individuals (age = 34.94 ± 8.0 years, height = 1.62 ± 0.06 m, weight = 61.1 ± 6.3 kg; mean

± SD). Possible candidates were excluded if they presented a recent osteoarticular and musculotendinous injury or surgery of lower extremities, a background of or signs of neurological dysfunction or medication which could affect motor performance and balance and individuals who had used unstable footwear (specifically Masai Barefoot Technology - MBT) prior to the study.

The study was conducted according to the ethical norms of the Institutions involved and conformed to the Declaration of Helsinki, with informed consent from all participants.

2.2 Instrumentation

The electromyographic activity (EMGa) of the gastrocnemius medialis (GM), tibialis anterior (TA), rectus femoris (RF) and biceps femoris (BF) muscles was monitored using the MP 150 Workstation model from Biopac Systems, Inc. (USA), with steel surface electrodes, TD150 model, with bipolar configuration and an interelectrode distance of 20 mm and a ground electrode. The rectus abdominis and erector spinae were not included as our findings related to short term changes showed that they did not play a significant role in reactive balance adjustments during perturbed stance.

The CoP displacement values were obtained using a force plate, model FP4060-10 from Bertec Corporation (U.S.A), connected to a Bertec AM 6300 amplifier, with default gains and a 1000 Hz sampling rate. The amplifier was connected to a Biopac 16 bit analogical-digital converter.

2.3 Procedures

2.3.1 Skin preparation and electrode placement

The subjects' lower limb skin surfaces were prepared to reduce electrical resistance to less than 5000 Ω and measurement electrodes were placed at muscle mid-belly of dominant limb according to anatomical references and fixed with adhesive tape.

2.3.2 Data acquisition

Each subject performed two tests: one standing barefoot and another with the unstable shoe, before and after an 8-week period. Subjects were instructed to stand relaxed, with feet comfortably spaced and arms at sides, and to look straight ahead to a target set 2 m away. Headphones were used to listen to music to mask any auditory cues and to distract the subject from consciously modifying her motion. A horizontal cable was attached to a pelvic belt worn by the subjects while they kept their bodies essentially straight. A backward force of 5% of body weight, measured with an isometric dynamometer, was applied to the cable for a random period of 3 to 10 seconds and then the cable was released (time zero, T_0). Test instructions to the subject were: "Stand still but compensate the force applied to the belt without moving your feet. I will let go at some point, but you will not know when. Do not move your feet, but keep your balance." The results obtained in a pilot study as to the inclination of the unstable shoe after applying the horizontal force demonstrated that the ankle dorsiflexion angle was not greater than 5º, which is not enough to produce changes in group Ia afferent feedback or in plantar and dorsiflexor muscle activity levels (Mezzarane & Kohn, 2007). Each subject performed two randomised series, one for each condition under study, of three trials each separated by 1-minute rest interval. As no noteworthy differences were verified between the first and the remainder of the trials of each series, the average values were used for analysis. Before data acquisition, all subjects were given time to become familiar with the test environment and were explained by a qualified instructor on how to use the unstable shoe, followed by approximately 10 minutes of walking, until the instructor felt they walked properly and were comfortable using the shoes (Nigg, Hintzen, et al., 2006).

The EMG signals were acquired according to a sample rate of 1000 Hz, pre-amplified at the electrode site, fed into a differential amplifier with an adjustable gain setting (12-500 Hz; Common Mode Rejection Ratio (CMRR): 95 dB at 60 Hz and input impedance of 100 MΩ), digitised and then stored in a computer for subsequent analysis based on the Acqknowledge software (Biopac Systems, Inc. USA). The gain range was set to 1000.

The muscle latency was detected in a time window from -450 to +200 ms in relation to T_0 (Santos, et al., 2009) using a combination of computational algorithms and visual inspection (Di Fabio, 1987). The latency for a specific muscle was defined as the instant lasting for at least 50 ms when its EMG amplitude was higher (activation) or lower (inhibition) than the mean of its baseline value plus 1 (one) standard deviation (SD) (Hodges & Bui, 1996), measured from -500 to -450 ms (Santos, et al., 2009). The signal was previously smoothed using a sixth order elliptical low-pass software filter of 50 Hz (Hodges & Bui, 1996).

To assess the level of muscle activity, signals were previously band-pass filtered between 20 and 450 Hz and integrated with 150 ms time windows. The integral of the EMG activity (Int_{EMGi}) of TA, GM, RF and BF was evaluated at two epochs relatively to T_0: 1) 50 to 200 ms (compensatory postural adjustments 1 (CPA1)), and 2) 200 to 350 ms (late compensatory postural adjustments 2 (CPA2)) (Latash, 2008; Santos, et al., 2009; Taube, et al., 2007). The Int_{EMGi} inside each epoch was corrected by the triple Int_{EMGi} from -500 to -450 (Santos, et al., 2009). As such, positive and negative values indicate increased and decreased muscle activation in relation to background activity. The Int_{EMGi} values were normalised according to the maximum voluntary contraction method (EMG_{norm}). After a warm-up consisting of 3 positioned at 90º manual resistance was applied for all muscles.

The CCI of agonist (GM, BF) – antagonist (TA, RF) muscles at joint and of dorsal (GM+BF) – ventral (TA+RF) muscle group levels was calculated using the following equations (Kellis, et al., 2003):

iv. CCI at joint levels:

$$CCI = \frac{EMGnorm_{TA,BF}}{EMGnorm_{GM,(BF+GM)} + EMGnorm_{TA,RF}} \times 100,$$

v. CCI at muscle group level:

$$CCI = \frac{EMGnorm_{TA+BF}}{EMGnorm_{BF+GM} + EMGnorm_{TA+RF}} \times 100.$$

This method provides an estimate of the relative activation of the pair of muscles as well as the magnitude of the co-contraction.

The R of the agonist-antagonist muscles at joint and muscle group levels were calculated as (Slijper & Latash, 2004):

a) R at joint levels

$$R = EMGnorm_{GM,(GM+BF)} - EMGnorm_{TA,RF},$$

b) R at muscle group level

$$R = EMGnorm_{GM+BF} - EMGnorm_{TA+RF}.$$

The acquired force time series of each trial were used to calculate the CoP fluctuation in the anterior-posterior (AP) direction as:

$$COP_{AP} = \frac{M_x}{F_z},$$

where M_x is the moment in the sagittal plane and F_z is the vertical component of the ground reaction force. A fourth-order, zero phase-lag, low-pass Butterworth filter with a cut-off frequency of 20 Hz (Winter, 1990) was applied to all CoP displacement time series. Only the CoP displacements in the AP direction will be reported, as the perturbations were induced symmetrically. The AP SD (SD_{AP}) and peak-to-peak ($P\text{-}P_{AP}$) distance of the CoP were measured in the following epochs: (1) +100 to +250 ms (CPA1); (2) +250 to +400 ms (CPA2). These values were selected to compensate the electromechanical delay (Howatson, et al., 2009) and were corrected as to basal values (obtained during unperturbed standing).

Following an initial evaluation, subjects in the EG were given a pair of the unstable shoes, being instructed to wear them as much as possible for at least 8 hours a day, 5 days a week, for 8 weeks, to obtain training effects (Nigg, Hintzen, et al., 2006; Ramstrand, et al., 2008; Ramstrand, et al., 2010; Romkes, et al., 2006). Also they received a guide on how to use the shoes. Participants in the CG were told to continue their normal activities and not begin any new exercise regime.

2.4 Statistics

The collected data were analysed using the software Statistic Package Social Science (SPSS) from IBM Company (USA). Differences between groups in terms of individual muscle activation, CCI and R at joint and muscle group levels, muscle onset and offset and CoP displacement, before and after the 8-week period, were analysed using the Mann-Whitney test. The Friedman ANOVA test was used to compare values obtained in the first and second evaluations in both groups and to compare CPA1 and CPA2 at the different levels in both groups.

3. Results

3.1 Influence on EMGa in CPA at individual, joint and muscle group levels

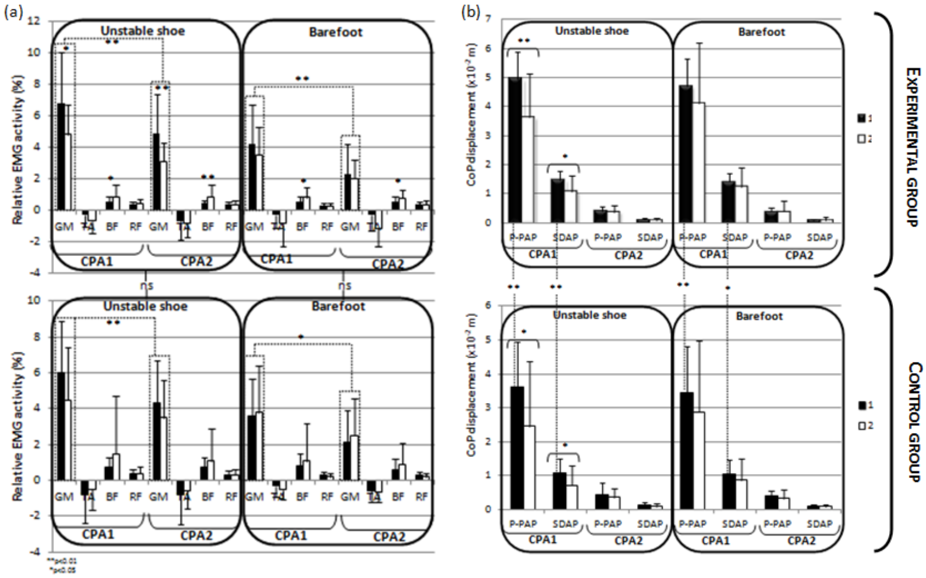

Figure 1: Representation of mean (bars) and SD (error bars) values of GM, TA, BF and RF EMGa (a) and CoP displacement values (b) during CPA, in barefoot and unstable shoe conditions, before (1) and after (2) 8 weeks of WUS by the experimental group and before and after the same period by the control group.

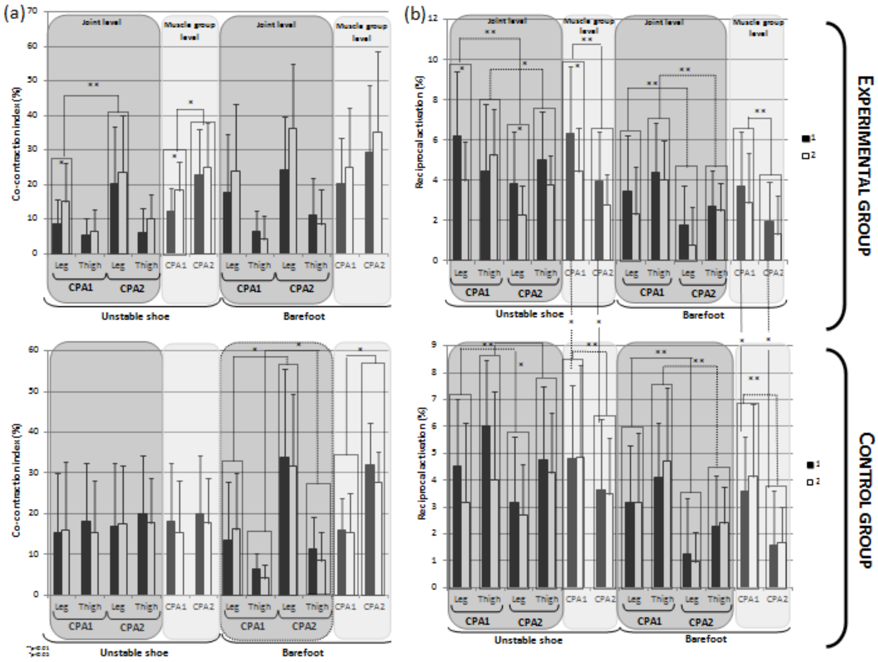

Figure 2: CCI (a) and R (b) values obtained during CPA before (1) and after (2) 8 weeks of WUS by the experimental group and before and after the same period by the control group.

Table 1: Proof values (p-values) obtained from comparisons made between first (1) and second (2) evaluations in the experimental group (EG) and control group (CG) and between groups. Only significant values are expressed numerically non-significant values are represented as ns.

Level	Epoch	Variable	Condition	p-value (1 and 2 comparisons)	p-value (CG and EG comparisons)	Condition	p-value (1 and 2 comparisons)	p-value (CG and EG comparisons)
Individual	CPA1	TA	Unstable shoe condition	EG: ns	ns in 1 and 2	Barefoot condition	EG: ns	ns in 1 and 2
				CG: ns			CG: ns	
		GM		EG: p=0.039			EG: ns	
				CG: ns			CG: ns	
		RF		EG: ns			EG: ns	
				CG: ns			CG: ns	
		BF		EG: p=0.028			EG: p=0.023	
				CG: ns			CG: ns	
	CPA2	TA		EG: ns			EG: ns	
				CG: ns			CG: ns	
		GM		EG: p=0.005			EG: ns	
				CG: ns			CG: ns	
		RF		EG: ns			EG: ns	
				CG: ns			CG: ns	
		BF		EG: p=0.006			EG: p=0.016	
				CG: ns			CG: ns	
Joint	CPA1	R leg		EG: p=0.023	ns in 1 and 2		ns in EG and CG	ns in 1 and 2
				CG: ns				
		R thigh		EG: ns				
				CG: ns				
		CCI leg		EG: p=0.028				
				CG: ns				
		CCI thigh		EG: ns				
				CG: ns				
	CPA2	R leg		EG: p=0.033				
				CG: ns				
		R thigh		EG: ns				
				CG: ns				
		CCI leg		EG: ns				
				CG: ns				
		CCI thigh		EG: ns				
				CG: ns				
Muscle group	CPA1	R		EG: p=0.028	1: p=0.04		ns in EG and CG	1: p=0.028
				CG: ns	2: ns			2: ns
		CCI		EG: p=0.011	1: ns			1: ns
				CG: ns	2: ns			2: ns
	CPA2	R		EG: ns	1: p=0.003			1: p=0.007
				CG: ns	2: ns			2: ns
		CCI		EG: ns	1: ns			1: ns
				CG: ns	2: ns			2: ns
CoP	CPA1	$P\text{-}P_{AP}$		EG: p=0.001	1: p=0.006		ns in EG and CG	1: p=0.004
	CPA2			CG: p=0.033	2: ns			2:
	CPA1	SD_{AP}		EG: p=0.023	1: p=0.01			1: p=0.005
	CPA2			CG: p=0.033	2: ns			2:
Muscle latency		TA offset		ns in EG and CG	ns in 1 and 2		ns in EG and CG	1: ns
								2: p=0.003
		GM onset						1: ns
								2: ns

WUS led to decreased GM activity and increased BF activity when WUS, and to an increased BF activity in the barefoot condition. No differences were observed between measurements either in the CG or between CG and EG. GM activity was higher in CPA1 in all evaluations (Figure 1, Table 1).

In Figure 2, it can be noticed an increase of CCI values in CPA1 at leg and muscle group levels after WUS only in the unstable shoe condition. In the CG there were no significant differences for these values. CCI was higher in CPA2 than in CPA1 at leg and muscle group levels when WUS for the EG, and at all levels in the barefoot condition for the CG (Table 1).

The R values (Figure 2) decreased at the leg and muscle group levels after the 8 weeks of WUS, also only in the unstable shoe condition and no differences were observed in the CG. Although the EG showed higher R values in the first evaluation at muscle group level than CG, no differences were observed in the second evaluation (Table 1). In both groups, R values were generally higher in CPA1 than in CPA2. However, at the thigh level the EG showed higher R levels in CPA2 in the first evaluation and in CPA1 in the second evaluation (Table 2).

3.2 Influence on CoP displacement in CPA

In both groups, the P-PAP and SDAP decreased in CPA1 in the second evaluation. However, the EG showed higher values of P-PAP and SDAP in CPA1 than the CG before training, which was not observed after the 8-week period of WUS (Figure 1 and Table 1).

3.3 Influence on muscle latency

No differences were observed in the EG after the 8-week period in the TA offset and GM onset. Statistically significant differences between the two groups were only found in the TA offset in the barefoot condition in the second evaluation (Figure 3 and Table 1).

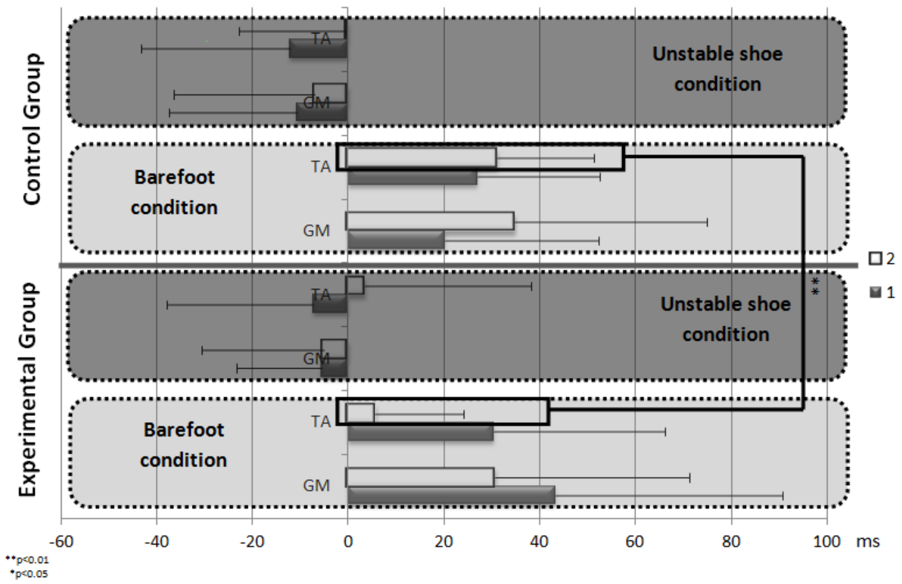

Figure 3: Onset and offset latency of leg muscles to an external perturbation before (1) and after (2) 8 weeks of WUS by the experimental group and before and after the same period by the control group, in barefoot and unstable shoe conditions. The muscle latency was only evaluated in TA and GM, as the main changes in muscle activity level, CCI and R occur at this level.

4. DISCUSSION

4.1 Influence on muscle activity at the individual muscle level

The results of this study demonstrate that WUS leads to long-term changes both when wearing the unstable shoe as well in the barefoot condition. Changes in agonist muscle activity (GM and BF) suggest that there is a transition between the work generated at the ankle to the knee/hip as a result of WUS. This transfer of work to larger muscle groups would allow muscles to work at a lower percentage of their maximum capacity, and therefore, optimise the energy consumption. This mechanism has been suggested to explain the higher activity of proximal muscles in relation to the more distal ones when walking at faster speeds (Chen, et al., 1997). The transfer of changes associated to WUS to the barefoot condition has not been found in measurements in other functional activities like standing (Sousa, Tavares, Rodrigues, et al., 2012) and walking (Stöggl, et al., 2010). Our findings suggest that there is a long-term transfer of changes associated with the unstable shoe condition to other conditions, like barefoot, in tasks associated to higher postural control demands. Like in other studies (Landry, et al., 2010; Ramstrand, et al., 2010), in spite of the changes mentioned, results were not statistically different between EG and CG.

Table 2: Proof values (p-values) obtained from comparisons made between CPA1 and CPA2 in first (1) and second (2) evaluations in the experimental group (EG) and control group (CG). Only significant values are expressed numerically and non-significant values are represented as ns.

Level	Evaluation	Variable compared	Unstable shoe condition p-value	Barefoot condition p-value
Individual	1	TA	EG: ns	EG: ns
			CG: ns	CG: ns
		GM	EG: p=0.003	EG: p=0.002
			CG: p=0.004	CG: p=0.003
		RF	EG: ns	EG: ns
			CG: ns	CG: ns
		BF	EG: ns	EG: ns
			CG: ns	CG: ns
	2	TA	EG: ns	EG: ns
			CG: ns	CG: ns
		GM	EG: p=0.002	EG: p=0.003
			CG: p=0.017	CG: p<0.0001
		RF	EG: ns	EG: ns
			CG: ns	CG: ns
		BF	EG: ns	EG: ns
			CG: ns	CG: ns
Joint	1	R leg	EG: p=0.001	EG: p=0.003
			CG: p=0.005	CG: p=0.003
		R thigh	EG: p=0.008	EG: p=0.004
			CG: p=0.008	CG: p=0.004
		CCI leg	EG: p=0.001	EG: ns
			CG: ns	CG: p=0.001
		CCI thigh	EG: ns	EG: ns
			CG: ns	CG: p=0.008
	2	R leg	EG: p=0.002	EG: p=0.003
			CG: p<0.0001	CG: p<0.0001
		R thigh	EG: p=0.01	EG: p=0.005
			CG: p=0.01	CG: p=0.001
		CCI leg	EG: p=0.004	EG: ns
			CG: ns	CG: p=0.001
		CCI thigh	EG: ns	EG: ns
			CG: ns	CG: p=0.013
Muscle group	1	R	EG: p=0.002	EG: p=0.003
			CG: p=0.01	CG: p=0.003
		CCI	EG: p=0.002	EG: ns
			CG: ns	CG: p=0.001
	2	R	EG: p=0.002	EG: p=0.004
			CG: p=0.011	CG: p<0.0001
		CCI	EG: p=0.003	EG: ns
			CG: ns	CG: p=0.001

In our study, differences at the individual muscle level between CPA1 and CPA2 were only observed at the ankle joint, suggesting a higher spinal and supraspinal modulation at this level. The maintenance of this difference after WUS suggests that the general patterns of postural reactions were preserved regardless of the adaptation mechanisms in terms of muscle activity level.

4.2 Influence on CCI and R levels at joint and muscle group levels

An increased leg CCI in CPA1 was observed after WUS for 8 weeks, in the unstable shoe condition. Previous research has shown that balance training leads to intensification of supraspinal induced pre-synaptic inhibition of Ia afferents (Gruber, et al., 2007; Taube, et al., 2007). The interval used to evaluate CPA1 (50-200 ms) included short latency reflexes (~50ms), but also long latency reflexes (~120ms) (Taube, et al., 2007). Taking this into account, the increase of leg CCI during CPA1 could be explained by an increased pre-synaptic inhibition as our results show that WUS did not lead to changes in short latency. However, future studies should evaluate the influence of WUS on the H-reflex. It has been hypothesised that some excitability in the segmental circuits of the antagonists may allow for their fast recruitment when necessary, such as in the maintenance of equilibrium during postural tasks (Lavoie, et al., 1997). Also, the increase of CCI could result from the need to reduce the degrees of freedom of body segments. Although in this study kinematic data were not acquired we can suggest, based on findings previews research during gait, that a decrease of kinematic variability should occur as a result of long-term WUS (Stöggl, et al., 2010). However, future studies should analyse kinematic data. Finally, the higher CCI value in CPA1 only occurred when WUS suggesting that there was an adaptation only to the unstable shoe condition, which is consistent with task-specific reflex (Taube, et al., 2007) and supraspinal (Schubert et al., 2008) adaptations.

The increase of CCI at the leg segment in the EG was associated with a decrease of R in the same segment when WUS. This reduction can be associated to the increased GM activity observed at the individual level since the strength of the disynaptic inhibition is related to the level of motor activity in the agonist (Lavoie, et al., 1997). In fact, it was verified that the strength of disynaptic inhibition is reduced during co-contraction of antagonist muscles compared with reciprocal activation (Nielsen & Kagamihara, 1992). Considering that reciprocal inhibition is stronger in tasks involving more joint movement (Lavoie, et al., 1997), the reduction of R obtained in our study could be related to the reduction of $P\text{-}P_{AP}$ and SD_{AP} after WUS. The lack of changes in the CG variables between the first and second evaluations indicates that changes at individual muscle activity, CCI and R values in the EG were related to WUS.

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